CLINICAL EVALUATION OF DARUHARIDRA (*Berberis aristata* DC.)-YASTIMADHU (*Glyeyrrhiza glabra* Linn.) EYE DROPS IN THE MANAGEMENT OF DRY EYE SYNDROME (SHUSHKASHIPAKA)

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Under the Board of Studies(Ayurveda)

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CERTIFICATE

This is to certify that the thesis entitled '**CLINICAL EVALUATION OF DARUHARIDRA (*Berberis aristata* DC.) –YASTIMADHU (*Glyeyrrhiza glabra* Linn.) EYE DROPS IN THE MANAGEMENTOF DRY EYE SYNDROME (SHUSHKASHIPAKA)'** which is being submitted herewith for the award of degree of Vidyavachaspati (Ph.D.) in the Late Vd. P.G. Nanal Department of Ayurveda of Tilak MharashtraVidyapeeth, Pune is a result of original research work completed by **Narayanam Srikanth** under my supervision and guidance.

To the best of my knowledge and belief, the work incorporate in this thesis has not formed the basis for the award of any degree or other similar title of this or any other University or examination body upon him.

(R. R.Padmavar)
Research Guide

Place:

Date:

Tilak Maharashtra Vidyapeeth, Pune

DECLARATION

I hereby declare that the thesis entitled as 'CLINICAL EVALUATION OF DARUHARIDRA (*Berberis aristata* DC.) AND YASTIMADHU (*Glyeyrrhiza glabra* Linn.) EYE DROPS IN THE MANAGEMENTOF DRY EYE SYNDROME (SHUSHKASHIPAKA)'completed and written by me has not previously been formed as the basis for the award of any degree or other similar title upon me of this or any other University or examining body.

(Narayanam Srikanth)
Research Student

Place: New Delhi

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ABSTRACT

Dry Eye Syndrome is a leading cause of ocular discomfort affecting millions of people. Dry Eye conditions are a spectrum of disorders with varied etiology ranging from mild eyestrain to very severe dry eyes with sight threatening complications. Dry Eye Syndrome is the most common eye disease, affecting 5 - 6% of the population. Further it became a significant public health problem distributed among 10% of the adult population and 18% of the elderly population. Dry Eye Syndrome (DES) is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tears film instability with potential damage to the ocular surface. It is accompanied by increased osmolality of the tear film and inflammation of the ocular surface. Based on the pathophysiology of tear film formation the classification of DES suggested by Holly and Lemp comprise Aqueous Tear Deficiency (ATD);Lacrimal surfactant (mucin) deficiency; Lipid layer abnormality; Impaired lid function or blinking; and Epithelio-pathy.

Ayurvedic literatures recount Dry Eye Syndrome (DES) or 'kerato-conjunctivitis sicca'(KCS) as *Shushkakshipaka, Parishuskha-netra, Ativishuskha-netra, Asrusravarahita-netra* and *Asnigdha-netra* indicative of dryness of eye due to deficiency in tear film components.

A critical interpretation of the terminology mentioned in different Ayurvedic texts while describing the disease reveals the role of tear film, other factors in patho- physiology of dry eye disease. The pre-corneal tear film has three layers, the outer lipid layer, the middle aqueous layer and the inner mucin layer, each has its own role to play viz. to retard the evaporation of aqueous layer, to increase surface tension so that the film is stable, to lubricate the eye lids, to supply atmospheric oxygen to corneal epithelium and it has anti-bacterial enzymes; laysozyme and lactoferin**.** Ayurvedic literatures recount the *tarpaka-kapha* as the essential factor attributed with functions and protective role identical to that of tear film.

Kapha is responsible for *sanigdhatwa*(lubrication) *sthiratwa* (structural and functional integrity of body systems) by virtue of its qualities like *gurutwa* and *snigdwatva*.

Tarpaka-kapha, one among the five varieties of *Kapha*, situated in head (*siras*)is responsible for the integrity of sense organs (*aksha-tarpana*). According to *Dalhana* the term *aksha* refers to sense organs such as eye. Collective function tear film components can be correlated with the function of the *tarpa-kakapha*.

Management strategies of Dry eye syndrome include mainly supplementation of tear preferably substitutes containing methylcellulose or carboxy-methylcellulose or identical substances which are viscous in nature**.** Preservatives used in formulations are known to cause dry eyes. All these drugs do not have any effect on basic path physiology and they provide only symptomatic relief**.** The tear stimulants such as cholinergic drugs increases the tear production from lacrimal gland by stimulating secretions, but not been used in clinical practice. Tear Preservation can be done by occluding the puncta or minimizing evaporation, But is useful as short-term measure to assess the effect of occluding puncta before resorting to permanent measures. All these drugs do not have any effect on basic path physiology and they provide only symptomatic ctions and inflammation. But discontinuation of antibiotics, steroids and all preservative-containing eye drops is mandatory for relief of symptoms and progressively improving the tear film and ocular surface. In view of the above, there is an urgent need to evolve safe and effective management approach to tackle symptoms, infections and underlying pathology

Owing to the above challenges of different conventional agents, it is at this juncture that the need for drugs/measures that could effectively tackle Dry eye syndrome. A vast number of indigenous drugs coupled with innumerable claims of their varied uses in alleviating wide range of ophthalmic affections calls for scientific validation for their attributes and principles.

Ayurvedic literatures record more than fifty ophthalmic plant drugs and more than forty metals minerals having diversified pharmacological actions on visual system and adnexa of the eye. **(**Srikanth, N., 2000. The Actions and Uses of Indigenous Ophthalmic Drugs, Chaukhamba Sanskrit Prathisthan, Delhi; Srikanth N, Ancient Ocular Therapeutics-An integrated approach., Ayur Medline Vol.I, April, 1999;SrikanthN,Hazra J., A Bird's eye view on single Metal & Mineral Ophthalmic drugs - An Ayurvedic Pharmacological basis, Sachitra Ayurved, July, 1999**)**

A critical review of classical literature and scientific studies is evident that the combination of ingredients viz. *Yastimadhu* (*Glyeyrrhiza glabra* Linn.) and *Daruharidra* (*Berberis aristata* DC.) certainly play a significant role in restoring the functions of tear film ,prevention of ulceration and related checking inflammatory process and contributory to the comprehensive management of Dry eye syndrome .

Ayurvedic literatures recount potential ophthalmic drugs for the management of surface inflammatory conditions of eye such as*sushkashipaka* or *parisushkanetra*a comparable with dry eye syndrome(DES) or Keratoconjunctivitis Sic ca(KCS) . These plants are attributed with Pharmacological actions such as *caksusya* (conducive to vision), *netrya* (conducive to adnexa of eye), *netraruja-hara* (analgesic ophthalmic action), *netra-sodhahara*(anti-inflammatory action) *netra-kanduhara* (anti allergic action), *vrana-ropana* (wound healing effect)b acked by scientific evidences.

Further scientific experimental studies also revealed antioxidant, anti-bacterial, antifungal, antioxidant which are contributory to comprehensive- management of dry eye disease by restoring the functions of tear film comparable to functional attribute – *tarpaka kapha*.

With this rationale and background, an Ayurvedic eye drops was developed for dry eye syndrome (DES) systematically following appropriate methods and parameters right from quality assurance of ingredients, formulation of standard operation procedures (SoPs) and also complying to the quality and safety standards of finished product for ophthalmic preparation as specified in Ayurvedic Pharmacopeia of India and Indian Pharmacopeia. (N. Srikanth,Arjun Singh,,Sharad D. Pawar ,S. N. Murthy and R.R. Padmavar. Development and Standardization of An Ayurvedic Herbal Eye Drops for Dry Eye Syndrome, World Journal of Pharmaceutical Research,06/2015; 4(6) 1034-1041.)

The ocular toxicity studies of standardized herbal eye drops revealed its safety on topical ophthalmic use. (N. Srikanth, Sharad D. Pawar, Arjun Singh, S. N. Murthy and R.R. Padmavar. Ocular Safety and Toxicity Studies of An Ayurvedic Herbal Eye Drop for Dry Eye Syndrome, European Journal of Biomedical and Pharmaceutical sciences, 06/2015; 2(3):679-687)

Further the antioxidant and anti-microbial property certainly contributes to effective symptom management and extenuation of basic pathology linked with tears component deficiency. (N. Srikanth, Sharad D. Pawar, Arjun Singh, S. N. Murthy and R.R. Padmavar.IN VITRO BIOCHEMICAL ASSESSMENT OF ANTIOXIDANT POTENTIAL OF AN AYURVEDIC HERBAL EYE DROPS FOR DRY EYE SYNDROME, European Journal of Pharmaceutical and Medical Research.2015,2(6), 261-266; N. Srikanth, Sharad D. Pawar, Arjun Singh, S. N.Murthy and R.R. Padmavar. ANTIMICROBIAL ASSAYS OF AN AYURVEDIC HERBAL EYE DROPS FOR DRY EYE SYNDROME, World Journal of Pharmacy and Pharmaceutical Sciences (Impact Factor: 5.21). 12/2015; Volume 4, Issue 12, 711-721)

Systematic and well-designed clinical studies study revealed statistically significant relief of subjective parameters and clinical symptoms of dry eye such as Blurred Vision, Feeling of dryness, Burning sensation, Foreign Body Sensation, Narrowing of aperture, Pricking pain, Redness, Rough Lids, Stuck eyelids .Adding to this the objective assessment of Tear film break-up time(TUBT),Schirmer-I test, Rose Bengal staining performed at baseline (0 –day) and 28th day has shown remarkable progress in terms of improving wetting of ocular surface and restoration of components of tear film functions. Besides this, the eye drops are well tolerated without complications, ADRs and AEs.

The eye drops containing *Daruharidra* (*Berberis aristata* DC.) & *Yastimadhu* (*Glyeyrrhiza glabra* Linn) developed rationally taking potential leads from codified Ayurvedic texts probably contribute by offering comprehensive management for dry eye syndrome .The scientific pre-clinical and clinical studies generated a perceptible evidence and revealed that the eye drop safe , well tolerable and clinically effective comparable to conventional control drug tear supplement-Carboxy methyl cellulose

Chapter-1
Introduction

Chapter-1: Introduction

1.1. Background: Dry Eye Syndrome (DES) is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tears film instability with potential damage to the ocular surface. It is accompanied by increased osmolality of the tear film and inflammation of the ocular surface. Based on the pathophysiology of tear film formation the classification of DES suggested by Holly and Lemp comprise Aqueous Tear Deficiency (ATD); Lacrimal surfactant (mucin) deficiency; Lipid layer abnormality; Impaired lid function or blinking; and Epithelio-pathy.

The Latin phrase 'kerato-conjunctivitis sicca'(KCS) indicates dryness and inflammation of the cornea and conjunctiva. There are many conditions which cause dryness of the eyes such as hypo function of lacrimal glands, mucin deficiency, conjunctival scarring etc**.** Although the typical patient of dry eyes is elderly or suffers from auto-immune disease, increasing number of patients do not fit this profile. Younger patients who work with computers can suffer from dry eyes more often than elderly patients. Dry Eye conditions is also aggravated in polluted conditions, dry weather, decreased ambient humidity as seen with air conditioning and indoor heaters. Ocular surface diseases can result from the abnormalities in one or more of the tear film components, ocular or systemic diseases, various drugs and even environment factors.

Dry Eye Syndrome is a leading cause of ocular discomfort affecting millions of people. Dry Eye conditions are a spectrum of disorders with varied etiology ranging from mild eyestrain to very severe dry eyes with sight threatening complications. Dry Eye Syndrome is the most common eye disease, affecting 5 - 6% of the population. Further it became a significant public health problem distributed among 10% of the adult population and 18% of the elderly population

Ayurvedic literatures recount Dry Eye Syndrome (DES) or 'kerato-conjunctivitis sicca'(KCS) as *Shushkakshipaka, Parishuskha-netra, Ativishuskha-netra, Asru sravarahita-netra* and *Asnigdha-netra* indicative of dryness of eye due to deficiency in tear film components.

1.2. Classical Review: In certain conditions, there is insufficiency of lubrication of eye and the conjunctiva becomes dry. Deficiency in any components of tear film, results in dryness of the eye, due to the appearance of dry spots on the corneal and conjunctival epithelium. Ayurvedic literatures vividly describe the conditions leading to dry ness of eye and recognized the role of tear components in maintaining surface ocular health. The role of *vata* and *pitta* doshas has been clearly dealt in the pathogenesis of the condition in different texts. Further, Ayurvedic texts clearly narrated several extrinsic, intrinsic, environmental factors including diet and lifestyle related attributes leading to causation ocular disease, the comprise;

- Sudden plugging into cold water after exposing oneself to Sun.
- Looking for a long time at distance objects.
- Constant looking at too minute objects.
- Improper sleeping habits such as day sleeping and night awakening.
- Exposure to dust, smoke, etc.
- Suppression of physiological urges like vomiting
- Excessive physical exertion
- Excessive exposure to fomentation
- Excessive smoking
- Social pathological factors such as worrying, anxiety, stress, strain, emotion etc.
- Unpleasant lasting environmental situations.
- Excessive sexual indulgence
- Ocular injuries and injuries to eye resulting from head injury (*Shirobhitapa* and *Shiroabhighata*).
- Excessive consumption of alcoholic (*madya*) and fermented sour liquids (such as *sukta,aranala*).
- Excessive consumption of *Kulatha* (*Dolichus uniflors*), *Masha* (*Casseolus radiata*), intake of sour food etc. (*Sushrutha samhita, UttaraTantra* 1/26; *Ashtanga Hridaya, Sutra Sthana* 2/3).

उष्णाभितप्तस्यजलप्रवेषात्दूरेज्ञणातृस्वप्नविपर्ययाच्च।

प्रसक्तसंरोदनकोपषोकक्लेषाभिघातादतिमैथुनाच्च।

शुक्तारनालम्लाकुलत्थमाषनिषेवणाद्वेगविनिग्रहाच्च।

स्वेदादथोधूमनिषेवणाच्चछर्दैर्विघाताद्वमनातियोणात्।

वाष्पग्रहात्सूक्ष्मनिरीक्षणाच्चनेत्रेविकारान्जनयन्तिदोषाः।। (सु.उ.1/26-27)

सिरानुसारिभिर्दोषैर्विगुणैरूध्वमागतैः।जायन्तेनेत्रभागेषुरोगाःपरमदारूणाः।। (सु.उ. 1/20-21)

Sushruta the contributor of the text *Susruta samhita* considered *Shushkakshipaka* as an individual disease and classified under *sarvagata-netraroga*, that can be manageable with pharmacological therapies (*asastra-krita*).Even though there is no separate entity such as *parishuskha-netra* in classics, authors of different texts mentioned the above condition while describing the *tarpana-kriyakalpa.* Further conditions like *ativishuskha-netra, asrusravarahita-netra, asnigdha-netra* are mentioned in *nibandha samgraha*, one of the commentaries on the text *Sushruta-samhita.*

According to *Susruta,Shushkakshipaka*, is characterized by difficulty in the eye lid movement ,pain while opening the eye (*sudarunamyatpratibodhaneca - krichronmilanam*),associated with rough and stiff lids (*darunaruksa-vartma*) and blurred vision (*vilokanecaaviladarsanamyat*) due to excessive vitiation of *vata*.

Vagbhata the contributor of text- *Astanga Hridya* has attributed involvement of *vata* and *pitta* doshas in the pathogenesis and described several additional symptoms such as pricking and cutting type of pain along with discharges. The eye lids become dry and hard making it difficult to open and close the eyes. There will be acute pain of suppuration and craving for cold application.

Further the text *Karala tantra* recounts excessive burning sensation (*daha*),producing closure and (*kunita*) roughness (*khara*) in eye lids, associated with difficulty in lid movement, blurred vision (*avilaeksana*) burning sensation etc. (KaralaTantra as quoted by *Madhukosa* commentary on *Madhava Nidana*. 59.17)

यत्कूणितंदारूणरूक्षवत्र्मविलोकनेचाविलदर्शनंयत्।
सुदारूणंयत्प्रतिबोधनेचशुष्काक्षिपाकोपहतंतदक्षि।। (सु.उ. **6/26)**

कूणितंखरवत्माक्षिकृच्छन्मीलाविलेक्षणम्।सदाहंसासृजाद्वाताच्छुष्कपाकाविन्तंवदेत्।।
- करालतन्त्र (मधुकोशमा.नि. **59/17)**

वातापित्तातुरंघर्षतादभेदोपदेहवत्।रूक्षदारूणवत्र्माक्षिकृच्छोन्मीलनिमीलनम्।
विकूणनविशुष्कत्वशीतेच्छाशूलपाकवत्।उक्तःशुष्काक्षिपाकोऽयम्
(अ.ह.उ. **15/16-17,**अं.स.उ. **18/14)**

1.3. Inter disciplinary understanding: A critical interpretation of the terminology mentioned in different Ayurvedic texts while describing the disease reveals the role of tear film, other factors in patho- physiology of dry eye disease. **(Table-1)**

Table-1. Ayurvedic terminology and its contemporary relevance in understanding the patho- physiology of Dry eye Syndrome

SNo.	Terminology mentioned in Ayurvedic texts for *Shushkakshipaka* (dry eye disease)	Contemporary relevance
1	*Shushkakshipaka*	Dry inflammation of eye due to tear component deficiency
2	*Parishuskhanetra*	Dry condition of eye possibly due to tear film disturbances
3	*Ativishuskha-netra*	Dryness of eye possibly due to tear film deficiency
4.	*Asrusravarahita-netra*,	Indicative of tear film deficiency in Aqueous Layer
5.	*Aasnigdha-netra*	Clear indicating of tear film deficiency in lipid layer and mucin layer

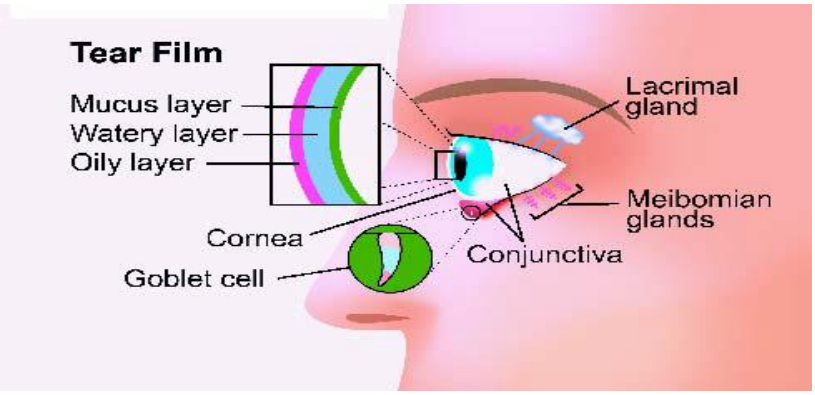
Tear film vis-à-vis *Tarpaka kapha* and its role in maintaining the surface environment and lubricating the eye: The pre-corneal tear film has three layers, the outer lipid layer, the middle aqueous layer and the inner mucin layer, each has its own role to play viz. to retard the evaporation of aqueous layer, to increase surface tension so that the film is stable, to lubricate the eye lids, to supply atmospheric oxygen to corneal epithelium and it has anti-bacterial enzymes; laysozyme and lactoferin**.** Ayurvedic literatures recount the *tarpaka-kaphaas* the essential factor attributed with functions and protective role identical to that of tear film.

Kapha is responsible for *sanigdhatwa* (lubrication) *sthiratwa* (structural and functional integrity of body systems) by virtue of its qualities like *gurutwa* and *snigdwatva*.*Tarpaka-kapha*, one among the five varieties of *Kapha*, situated in head (*siras*)is responsible for the integrity of sense organs (*aksha-tarpana*). According to *Dalhana* the term *aksha* refers to sense organs such as eye. Collective function tear film components can be correlated with the function of the *tarpa-kakapha*.**(Table-2, Fig-1)**

Table -2.Tear film vis-à-vis *Tarpaka-kapha* and its role in maintaining the surface Ocular health

Tear Film Components	**Mucin Layer**	**Aqueous Layer**	**Lipid Layer**
Source	***Conjunctival goblet cells***	***Main and accessory lacrimal glands***	***Meibomian glands***
Function	• Formation of glycocalyx renders the ocular surface hydrophilic. • Allows the viscosity of tears to change as per the shear rate of blinking. • Maintains the dioptric integrity of the tear film in the inter-blink interval. • Prevents adhesion of foreign bodies to the ocular surface. • Heavily glycosylated proteins, mucins, Immunoglobulins and antimicrobial proteins	• Creating the proper environment for the epithelial cells. • Provides essential nutrients and oxygen to the cornea. • Allows cell movement over the ocular surface. • Provides many of the growth factors necessary for ocular surface health. • Contains lysozyme, which is an anti-bacterial substance.	• Prevents the evaporation of tears. • Enhance stability of the tear film.

Fig-1.Tear Film Components –At a glance



1.4. Modern Review: Kerato-conjunctivitis sicca (KCS), or dry disease, is one of the most common complaints seen by ophthalmic specialists. In the current scenario of ageing population and increasing environmental factors it is becoming even more prevalent. Dry eye is not a trivial complaint. The symptoms cause significant discomfort and substantially reduce the sufferer's quality of life.

Normal sight is dependent on moist ocular surface. This moisture is maintained by a complex interplay of various factors, sufficient quantity of tears, a normal composition of the tear film, normal lid closure, and regular blinking of the lids. The tear film and the ocular surface form a stable system, which can lose its equilibrium Through numerous disturbing factors. The understanding of the causes and pathology of the dry eye has improved significantly in the recent years. Dry eye is a multifactorial disease the exhibits primary and secondary changes in the ocular surface.

Prevalence: Data on the epidemiology of dry eye is sparse even in the western literature. Adding to the confusion is the difference in the definition and inclusion criteria in different studies. Available data suggests that it is a significant problem in the older age group. In a community study in Sweden the prevalence rate of 15% was found in the general population aged 55-72 years.

This was done on the basis of symptoms of dry eye disease and positive findings on Schirmer's test, tear film break-up time, or rose Bengal staining. A recent Japanese study revealed a 17% rate of positive symptom of dry eye. Most other studies reveal a prevalence rate of between 11 and 17%. These studies found that symptoms of dry eye disease are more frequent in individuals above 50 years of age, however, they found no association of symptoms with sex or age.

The prevalence of dry eye disease in the community may increase in the future as the proportion of individuals over the age of 60 years of age grows. It would thus be fair to state that the individual ophthalmologist has to acquire the knowledge to both manage and educate the patients about the condition in the best possible way.

Definition of dry eye disease: The modern definition of dry eye disease is based on the concept of the three layers of the tear film devised by Holly and Lemp. Also, secondary factors such as pathological changes to the eyelids, cornea, or conjunctiva, can themselves disturb the normal function of the tear film. Neurotransmitters, hormones, and immunological processes play an important role in the regulation of tear production by the lacrimal gland. Various environmental factors like contact lenses, pollution, working at video display terminals can affect the tear film.

This multiplicity of causes and effects makes a global definition difficult. However, the following definition has been proposed: Dry eye is a disease of the ocular surface attributable to different disturbances of the natural function and protective mechanism of the external eye, leading to an unstable tear film during the open eye state. Recent studies have shown that immunologic changes play a role in the pathogenesis of the dry eye even in post infectious and age-related conditions. In addition to the term dry eye, which is established worldwide, the term ocular surface and tear disorder has been suggested.

Clinical Anatomy and Physiology: The lacrimal system produces a tear film that allows the ocular surface to function normally. A smooth, refractive surface and resistance to disease depend on a healthy tear film.

Secretory System: Tear Secretion comes from the lacrimal gland. The efferent nerve supply to the lacrimal gland is cholinergic. Drugs that inhibit cholinergic activity, therefore, inhibit tear secretion and often cause dry eye syndrome.

Perhaps more important to maintenance of the tear film are the basic secretors. The three layer tear film has numerous contributors. The sebaceous Meibomian glands produce the outermost lipid layer. The glands of Zeis at the palpebral margin of each eyelid and the glands of Moll at the roots of the eyelashes also contribute to this layer.

The accessory lacrimal glands of Krause and Wolfring are responsible for producing the middle layer of the tear film.

The inner layer of the tear film is a mucoid layer of polysaccharide (sialomucus) derived primarily from the conjunctival goblet cells located in the fornices. Also contributing to this layer are the tarsal crypts of Henle and limbal glands of Manz.

The basic secretors together produce the continuous flow of tears that bathe the globe. They have no confirmed afferent nerve supply, and their output decreases with age.

Distribution System: The distribution system for the tear film consists of the eyelids and the tear meniscus along the lid margins in the open eye. Each blink compresses the superficial lipid layer. The mucous layer acts as a scavenger to pick up any lipid containing debris and carry it to the fornices. As the eyelid reopens, a new tear –film layer is spread across the ocular surface. Inadequacy of any layer of the tear film increase its instability and may accelerate tear breakup time (BUT).

The distribution system of the lids also act as a pumping mechanism to draw tears into the excretory system.

Excretory System: Blinking is an important factor in tear distribution and also plays a pivotal role in tear drainage. Crucial to proper lacrimal excretory function is the punctum, the entry point for lacrimal drainage. Proper tear elimination requires that the punctum be apposed to the globe.Spontaneous blinking replenished the fluid flim by pushing a thin layer of fluid ahead of the lid margins as they come together. The excess fluid is directed into the lacrimal lake - a small triangular area lying in the angle bound by the innermost portions of the lids. Tears are drained from the lacrimal lake by the lacrimal canalculi via the nasolacrimal duct, and then drained over the nasopharynx and oropharynx to be swallowed. The drainage pathway may account for up to 90% of the fate of tears. The remainder evaporates. Thus, the act of blinking exerts a suction – free force action in removing tears from the lacrimal lake and emptying them into the nasal cavity.

Tear Fluid Composition: The tear fluid is found to be composed of three protein fractions: albumin, globulin and lysozyme. The immunoglobulins found in normal tear fluid are IgA, IgG and IgE. IgA predominates in the secretory form, IgE levels increase in patients with allergic conjunctivitis, and IgM is found in tears of patients with acute infections. Lysozyme may act synergistically with IgA in causing lysis of bacteria. Tears also contain lactoferrin, which has some antibacterial effect.

Tears: vital statistics

- Average glucose concentration of the tears is 2.5 mg/dl

- Average tear urea level is 0.04 mg/dl
- Electrolytes such as K^+, Na^+ and Cl^- occur in higher concentrations in the tears than in the blood
- Average pH of the tears is 7.25
- Osmolality is 309 mosm/liter (hypertonic in patients with the dry eye syndrome)
- Surface tension of the tear film is 40-72 mN/m
- Refractive index of the tear film is 1.336

Under normal conditions, the tear fluid forms a thin layer over the cornea and conjunctiva, this is known as the pre ocular tear film. The pre ocular tear film measure 8µm thick nad cover the corneal and conjunctival epithelial.

The pre-ocular tear film acts as an important component of the ocular defense mechanism.

1) It makes the cornea a smooth optical surface.
2) It helps to wet the cornea and the conjunctiva and prevents them from drying.
3) It flushes out the debris and organisms from the corneal surface.
4) It has bactericidal properties due to the presence of lysozyme, lactoferrinn and betalysin.
5) Immunoglobulins (IgA) and specific anti-bodies in the tears defend the eye against external infections.
6) Frictional trauma between the tarsal and the bulbar conjunctiva and cornea is minimized by the lubricating action of the tear film.
7) It enables the anti-inflammatory cells to reach the injured areas of the cornea and the conjunctiva.
8) It provides the epithelial cells with glucose, oxygen and growth factors.

Functions of Pre-ocular Film

Outer (Lipid) layer: Its principal function is to retard the evaporation of the tear film and enhance stability. A deficient lipid layer due to lack of secretion of the Meibomian glands occurs in patients of dry eye.

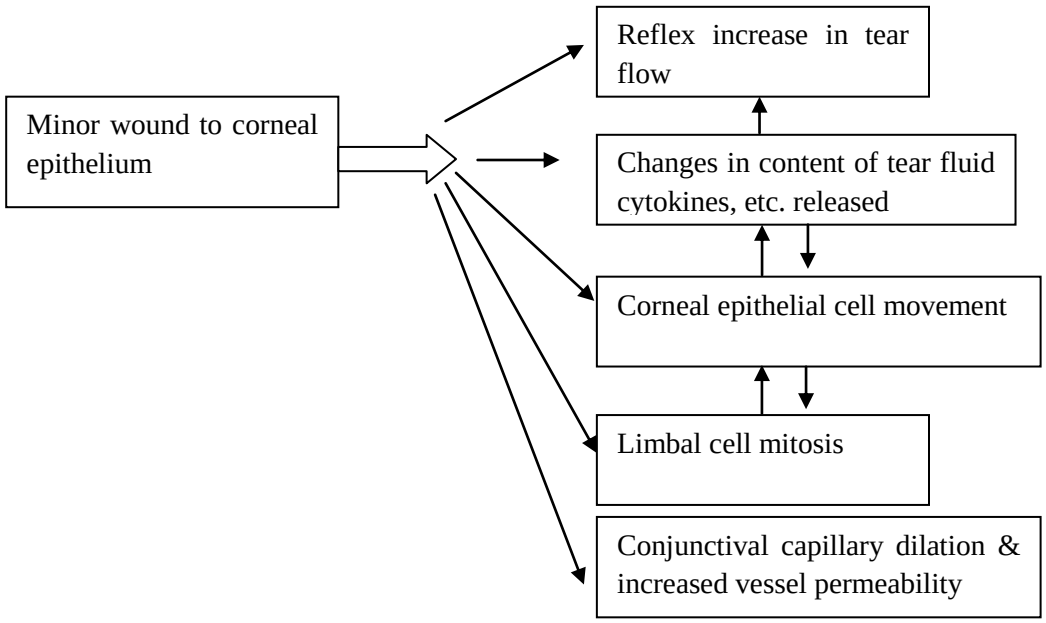
Middle (Aqueous) layer: The aqueous layer transports all the water soluble nutrients, Aqueous Tear Deficieny (ATD) is the main cause of dry eye syndrome.

Inner (Mucin) layer: The innermost mucin layer of the tear film forms a highly hydrophilic wetting surface over the hydrophobic epithelial cell membranes of cornea and conjunctiva. The mucus also reduces the surface tension between the lipid layer of the tear film and the water layer , thus contributing to the stability of the tear film.

With improving understanding of the interaction at the ocular surface, we now realize that the tear film, corneal epithelium, conjunctive, the lacrimal glands, and the eyelids act as a functional unit.

All the components of the functional unit are in anatomic continuity and share feedback mechanisms. This connectivity causes them to react to a stimulus as a single unit. This is exemplified by the simultaneous reaction elicited amongst the various components by a single insult (**Table -3.)**

Fig-2.Dry Eye-Simultaneous reaction elicited amongst the various components



A classification of Dry Eye Syndromes based on the pathophysiology of tear film formation was suggested by Holly and Lemp which is as follows;

1. Aqueous Tear Deficiency (ATD)
2. Lacrimal surfactant (mucin) deficiency
3. Lipid layer abnormality
4. Impaired lid function or blinking
5. Epitheliopathy i.e. a primary ocular surface abnormality leading to non-wetting.

1. Aqueous Tear Deficiency (ATD)

This is the commonest cause **of** Dry Eye Syndrome. The disease due to ATD is also called as Keratoconjunctivitissicca. Lack of the tear proteins makes the eye prone to infections and the consequent complications. It can be due to various conditions. The deficiency of aqueous tear layer can be due to

a. **Senile or idiopathic atrophy of lacrimal gland.**
b. **Menopause**
c. **Atrophy or hypofunction of the lacrimal gland associated with autoimmune diseases like Sjogren's syndrome.**

Sjogren's syndrome is a chronic, slowly progressing auto-immune disease characterized by lymphocytic infiltration of the exocrine glands resulting in xerostomia and dry eyes. The disease can be seen alone (primary Sjogren's disease) or in association with other auto-immune diseases (Secondary Sjogren's syndrome). The disease predominantly affects middle aged women (female to male ratio of 9:1).

Pathology: In the eye, the conjunctival epithelium shows a decrease in the goblet cell density, followed by squamous metaplasia and in later stages, keratinization.

Many patients with ATD have either circulating antiantibodies, including antinuclear antibody (ANA) or SS antibodies (SS-A and SS-B). The presence of these antibodies has been correlated with the severity of symptoms and ocular surface changes, including a higher incidence of sterile and bacterial keratitis. There may be an enlargement of the

salivary and lacrimal glands, owing to underlying systemic diseases such as leukemia, lymphoma and sarcoidosis (Mikulicz syndrome) with dry eyes.

Other rare causes of aqueous tear deficiency include aplasia or hypoplasia of the lacrimal gland (alacrimacongenita, Bonnevie-Ullrich syndrome), Anhydrotic ectodermal dysplasia, aplasia of lacrimal nerve nucleus, congenital familial sensory neuropathy with anhydrosis, familial autonomic dysfunction (Riley –Day syndrome), Holmes-Adie syndrome, multiple endocrine neoplasia. Hematopoietic and reticuleondothelial disorder like Felty's syndrome, malignant lymphoma, thrombocytopenic purpura, hemolytic anemia, hypergammaglohulinemia, Waldenstrom'smacroglobulinemia, and hepatic disorders like chronic hepatitis and primary biliary cirrhosis may also be associated with dry eyes.

Skin diseases like cicatricialpemphigoid, erythema multiforme (Stevens-Johnson syndrome), exfoliative dermatitis, scleroderma and epidermolysisbullosa reduce aqueous tear flow due to scar formation.

2. Lacrimal surfactant (Mucin) Deficiency:

Instability of the tear film due to mucin deficiency results from goblet cell dysfunction. Deficiency of the mucin layer decreases the wettability of the ocular surface leading to instability of the tear film and decreases in the tear film break-up time.The various causes are:

a. Traumatic destruction of conjunctiva
b. Vitamin A deficiency
c. Infections like diphtheritic keratoconjunctivitis, trachoma.
d. Mucocutaneous disorders like ocular pemphigoid, erythema multiforms, Steven-Johnson's syndrome.
e. Chemical, thermal and radiation injuries of the conjunctiva.
f. Drug induce: e.g. sulfonamides, epinephrine.

3. Lipid Layer Abnormality

a. Chronic blepharitis
b. Acne rosaecea

4. Impaired Lid function or Blinking

Shear forces produced by the moving lids plays a vital role in the maintenance of a normal tear film. Compromise of the lid globe contact or an abnormality of the normal blinking process can adversely affect the tear film stability and turnover.

Exposure keratitis is a condition where part of the ocular surface constantly gets exposed either due to neuroparalytic conditions or distortion of the eye lids. The drying of the tear film, causes inflammation and finally infection of the ocular tissue.

Neuroparalytic lesions of the following structures leads to impaired blinking

a) Cranial nerve VII (facial)
b) Greater superficial petrosal nerve
c) Sphenopalatine ganglion
d) Cranial nerve V (trigeminal)

5. Epitheliopathy:

Electron microscopy studies appear to indicate an intimate relationship between the microvilli of the corneal epithelium and the mucous layer. The tear film becomes thin and retracts near areas of epithelial irregularity. These areas are characterized by a drastic reduction in the BUT and in some areas permanently dry spots are formed.

6. Other causes of Dry Eye Syndrome

A. Drug induced-Drugs that decrease tear production

Antimuscarinics	Atropine Scopolamine
Antihistamines	Numerous
Anaesthetics **Enflurane** **halothane**	Nitrous oxide
Hypnotics	Nitrazepam
Phenothiazines	Numerous
Psychotropics Clomipramine	Diazepam
Others	Phenazopyridine

B. Visual Display Terminal Syndrome (VDTS)

An important cause of dry eyes in patients is use of contact lenses and computers. Many studies have shown that computer screens kept at or above the level of the eyes enhance the evaporation of the tears. This is because the palpebral fissure is widened and due to decrease in blink rate.

C. Contact Lens

Use of Contact lenses also contribute to the development of dry eyes. Rigid lenses disrupt the lipid layer enhancing the evaporation of the tear film while soft contact lenses actively deplete the rear layer to maintain their hydration level. Contact lenses also decrease the corneal sensation, a factor which may be necessary for the year secretion.

Clinical Features: There are many conditions which cause dryness of the eyes. Hypofunction of lacrimal glands (eg. sjogren's syndrome, sarcoidosls, lymphoma, leukemia amyloidosis), mucin deficiency (e.g. vitamin A deficiency), conjunctival scarring, (e.g. trachoma, stevans Johnson syndrome, pemphigold, chemical burns, chronic bacterial or viral conjunctivitis, irradiation and miscellaneous causes such as mumps, deficient blinking etc.).

Clinical Manifestations of dry eye may be trivial like itching or burning to serious one like blindness. The spectrum of complaints found in dry eyes in order of frequency include foreign body sensation (gritty feeling), excessive secretion, burning, redness, photophobia, blurred vision, itching, pain and inability to tear in crusting of eye lids and sticking.

This is commonly seen in the morning on waking up. Nonspecific symptoms include headache, include headache, heaviness of eyes or tiredness. Fatigue may be experienced while reading or watching TV. Occasionally patient may complain of excessive watering or mucous secretion due to imbalance of tear constituents.

Symptoms:

- Foreign body or sandy sensation
- Ocular irritation, itching and burning
- Blurred vision and photophobia
- Heaviness of eyelids, pain, redness
- Excessive mucous secretion

Dry eyes can be associated with indoor heating systems and air conditioning systems because of lower levels of relative humidity

The symptoms tend to be worse towards the end of the day with prolonged use of the eyes.

Some patients may complain of excessive tearing. This could possibly be due to reflex secretion in response to irritation in cases where cause is other than aqueous deficiency.

Signs: The tear film may show mucus debris and string of mucoiddischargs. The bulbar conjunctiva may lose its normal luster and may be thickened, edematous and hyperaemic or show slight anterior folding.

The picture may be of a non specific chronic conjunctivitis with thick enlarged mucus strands. In the fornix of the conjunctiva, these threads form owing to a slow tear flow and partly because of the increased number of the desquamated epithelial cells. The eyelids may frequently show concomitant evidences of chronic blepharitis and blepharospasm.

In most advanced states, filaments and mucus plaques are seen. Corneal mucus plaques may form due to abnormally viscid mucus. Marginal or paracentral thinning and even perforation can occur in advanced status. Inadequate and infrequent blinking may be seen in neuro-ophthalmic conditions.

Diagnostic Tests: An appropriate choice of test helps the clinician to arrive at an accurate diagnosis as well as for individualization of the therapy.

1. **Basic Secretion Test:** The purpose of this most commonly used test is to measure the basal secretion by eliminating reflex tearing. Topical anesthetic is instilled into the conjunctiva and a few minutes allowed passing until reactive hyperaemia has subsided. Any residual anesthetic should be wiped from the conjunctival sac using a sterile dry cotton tipped applicator. The room is darkened and the procedure is the same as in the Schirmer test I. Interpretation of the results is also similar. The difference between the results of this test and tose of the Schirmer test I is a measurement of reflex secretion. Materials used are the Schirmer strip and any anaesthetic like proparacaine 0.5%.Less than 5 mm of wetting on the basic secretion test ensures a diagnosis of hypo-secretion whether it results from an actual deficiency of basic tear film secretion or a peripheral sensory fatigue due to decreased inputs from the lacrimal nucleus can be determined by the schirmer's II test.

   Schirmer's Test I: Test secretion may be divided into basal and reflex secretion.The average basal tear volume is from 5-9µl with a flow rate of 0.5-2.2 μl/min..The purpose of this test is the measurement of the total (reflex and basal) tear secretion. To minimize reflex tearding, the eyes should not be manipulated before starting this test. There is no contraindication to this test. The materials used are commercially available Whatmann No. 41 filter paper strips 5mm x 30 mm, known as Schirmer tear test filter strips. The patient's is seated in a dimly lit room, and the filter paper strips are folded 5 mm from the end.

   The folded end is placed gently over the lower palperbral conjunctiva at its lateral one-third. The patient keeps the eyes open and looks upward. Blinking is permissible. After 5 minutes the strips are removed and the amount of wetting is measure from the folded end of the strips are completely wet before 5 minutes, they may be removed prematurely.

Measurements greater than 30 mm at 5 minutes indicate that refluxtearing is intact but not controlled and, therefore, are of little diagnostic value. Between 10 and 30 mm of tears secretion may be normal, or basal secretion may be low but compensated for by reflex secretion. Values less than 5 mm on repeated testing indicate hyposecretion of basic tearing. The practical significance of this test appears to be the confirmation of hyposecretion when less than 5 mm on wetting occurs. There is a 15% chance of diagnostic error in the test. To differentiate between basic and reflex tearing, the basic secretion test should be performed.

2. **Schirmer's Test II:**The purpose of this test is to ascertain reflex secretion. The procedure is similar to the basal secretion test, but after the strips are installed, the unanesthetized nasal mucosa is irritated b rubbing with a dry cotton-tipped applicator. The amounts of wetting o the filter paper is measured after 2 minutes. Less than 15 mm of wetting indicates failure of reflex secretion. Since this failure is not of major clinical consequence, the test is seldom used. Contraindications are the same as for the basal secretion test or the presence of nasal pathologic condition. Materials used are the same as for basal secretion test.

3. **Rose Bengal staining:** The purpose of this test is to ascertain indirectly, the presence of reduced tear volume by the detection of damaged epithelial cell. The eye is anesthetized topically with preparacaine 0.5%. Tetracaine or cocaine may give false positive tests because of their softening effect on corneal epithelium. One drop of 1% rose bengal solution or a drop from a saline water rose bengal strip is instilled in each conjunctival sac. Rose bengal is a vital stain taken up by dead and drying cells in the exposed interpalpebral area. This test is particularly useful in early stages of conjunctivitis sicca and keratoconjunctivitissicca syndrome. A positive test will show triangular stipple staining of the nasal and temporal bulbar conjunctiva in the inter-palpebral area and possible punctuate staining of the cornea, especially in the lower two thirds. False-positive staining may occur in conditions such as chronic conjunctivitis, acute chemical conjunctivitis secondary to hair spray use and drugs

such as tetracaine and cocaine, exposure keratitis, superficial punctuate keratitis secondary to toxic or idiopathic phenomena, and foreign bodies in the conjunctiva.

4. **Fluoresce in Dry Test:** This is a test for the stability of the tear film. After a certain time interval following blinking, the tear film normally ruptures and forms dry spots. Increased Meibomian gland secretion possibly may act to decrease tear film stability. Deficiency of mucin and acqueous tears will also decrease the tear film stability and shorten the time interval between the opening of the eye and the appearance of dry spots.
5. **The technique of tear film break-up time (BUT):** No anesthesia is used. 1% flourescein is installed in the lower cul-de-sac, or a dry fluorescein is touched to the inferior fornix with the patient looking up. The cornea is scanned under low slit lamp magnification using a blue cobalt filtered light. The patient is instructed to blink once or twice and then stare straight ahead without blinking. The time of appearance of the first dry spot formation (small black spots within the blue-green field) from the last blink measures the tear film BUT.

   Interpretation of BUT: A wetting time greater than 20 seconds reflects a normal tear film stability. BUT averages between 25 to 30 seconds in normal individuals. Women tend to have shorter BUT than men. Also BUT is less in elderly. There is also a correlation between BUT and the width of the palpebral fissure. BUT less than 10 seconds indicates significant tear film instability. The greater the number of dry spots, the more unstable the tear film. Fluorescein staining can be graded from 0-3. 0- no staining of corneal epithelial surface.

   1- mild staining occupying < 1/3 of corneal epithelial surface. 2- moderate staining occupying < ½ of corneal epithelial surface. 3- severe staining of > ½ of the corneal epithelial surface.

   The consistent appearance of dry spots in one area indicates an anatomic surface abnormality in that area. Abnormal BUT is invariably seen in clinically significant sicca and mucin deficient syndromes.

Precautions: Local anesthetic or tonometry prior to testing or holding the lids apart with the fingers, will give false-positive testing because these manipulations decrease the BUT.

Recently a more sophisticated tear film integrity test used is the non-invasive break-up time. This assessment involves projecting a fine grid onto the corneal surface and observing the first destruction of the pattern. The normal value of NIBUT may be as long as 40 seconds.

6. Other tests

Practical Double Vital Staining for Ocular Surface Evaluation: This is an innovation over the two tests, normally rose bengal staining and fluorescent staining of eye, as part of the diagnosis of dry eyes. Instead of two separate tests, this test uses both fluorescein and rose bengal. This is stored at 4 degrees centigrade in 5 ml bottle. A micropipette with a disposable tip is used to deliver a 2 μl drop into the lower cul-de-sac without anesthesia. Each tip is used for one eye only to prevent possible contamination. The staining patterns are observed by slit lamp. BUT is measured at the same time. The volume of 2 μl is standardized because the capacity of the cul-de-sac is 7 μl, as a result of which, there is no overflow of solution. The advantage of this technique is that there is no irritation because of low volume and preservative free mixture. The side effects of conventional tests such as irritation caused by over doses or preservatives, dyeing of eye lid by overflow and complicated techniques which include 2 separate staining procedures are avoided. The results are reproducible because of its fixed concentration and volume of the dyeing. Cross contamination is eliminated because of the disposable tip. This technique is strongly recommended for routine testing as its helps to detect subtle ocular surface abnormalities such as mild dry eye, the signs and symptoms of which vary and are difficult to detect.

Corneal Residence Time Test or the Tear Clearance Rate (TCR): Several methods can be used to measure the time that pre-ocular tear film and topical eye drops remain resident on the cornea. In patients who have a normal lacrimal drainage system but abnormalities in tear production, the decay time of an indicator such as flourescein or a radioisotope can be accurately measured. In patients with dry-eye syndrome, then

residence time of flourescein or a radioisotope is prolonged because of a decrease in the turnover of tears. The flourescein concentration can be detected by clinical observation or, more accurately, by flourophotometry, the radioisotope can be measured by gamma camera. These procedures remain research tools, but may be used in certain patients with dry-eye syndrome. Other methods also are helpful in measuring the residence time of certain drugs.

Tear Function Index: Tear Function Index is diagnostically superior to other tests in identifying dry eye and distinguishing dry eye that is associated with Sjogren's syndrome.

TFI is the combination of both tear secretion and drainage test. The Schirmer's test value with anaesthesia and the tear clearance rate are measured 5 minutes after instilling 10 µl drop of 0.5% flourescein and 0.4% oxybuphrocaine. A standaradSchirmer test strip is then placed for another 5 minutes into the conjunctival sac. The length of the wet portion is measured and the intensity is compared with standard strip colours. The TCR is determined by the rate at which the colour of fluorescent dry fades and is graded as 1, ½, ¼,…….1/64, 1/256. The TFI is defined as a value of Schirmer's test with anaesthesia divided by TCR.

Interpretation: The higher the value the better the ocular surface conditions. Values below 96 are highly suggestive of dry eye and values below 34 are highly associated with Sjogren'ssyndrome.The variations in the forces affecting tear drainage (i.e. gravity, siphonage, capillary attraction and muscular activity) is reflected in the values of the Schirmer test with anesthesia and the TCR. For example, if these forces are strong, the tears will drain more quickly into the nose, resulting in low Schirmer and high TCR values. Conversely, weak forces yield high Schirmer and low TCR values. Although we cannot measure tear secretion independently and directly, the combination of the Schirmer and the TCR tests reflects secretory and drainage conditions. The TFI incorporates both of these test and eliminates the influence by the forces of tear drainage. Because the principal influence on tear dynamics is tear secretion, which is reflected in both the Schirmer and TCR tests, and because the secretory ability differs between

normal subjects and patients with dry eye, the TFI can thus differentiate those with normal and dry eyes.

Tear Film Osmolality Test : For this test, tear samples are collected with hand-drawn micropipette from the inferior marginal tear strip without disturbing the ocular surface, and tear osmolarity is determined by a freezing point depression osmometer. Normal tear film osmolrity is 295 to 309 mosm/litre. Elevation of tear osmolarity suggests dry eyes.

Tear Lactoferrin Test: There is a decrease in lactoferrin levels in the reflex tear secretion of dry eyes. The test include Lactoplate, a radial immunodiffusin assay and an ELISA based test requiring only 10-15 minutes.

Test Lysozyme Test: Tearlysozyme is a useful measure of the aqueous tear secretion since it constitutes nearly ¼ of the total tear secretion proteins.

Patients with Sjogren's syndrome have decreased lysozyme production. Several methods-microbiological, turbidimetric, and spectrometric assays – are used to determine the level of lysozyme in tears. Normal tear lysozyme levels are between 2 and 4 mg per ml.

A Qualitative Mucous Assay

This test may be performed to determine the presence of mucus. Cotton strips 3 mm X 10 mm are placed in the inferior cul-de-sac of the unanesthezied eye for 5 minutes. Each strips is then placed on a glass slide and stained with PAS reagent. Color change is noted 1 minute later and compared with a sample from a known normal subject. If adequate mucus is present the strip will show a positive PAS reaction, turning dark purple. In the absence of mucus the reaction is negative. This test may be meaningful only in those eyes containing at least some tear film.

Conjunctival-Biopsy: The test may be done to ascertain the presence or absence of mucin- producing goblet cells. Four percent cocaine solution on a cotton tipped swab is applied directly to the lower nasal fornix, an area containing the highest population of goblet cells. After 60 seconds a conjunctival sample is obtained and subjected to acid Schiff (PAS) stain. In a mucin-deficient state goblet cells are markedly diminished.

Impression Cytology: This is sophisticated diagnostic strategy for confirming dry eye states. Conjunctival impression cytology is performed to determine goblet cell density of the bulbar or palpebral conjunctiva. A strip of filter paper is gently pressed against the bulbar or palpebral conjunctiva with a glass end. Following staining with Schiff's agent and counter staining with haematoxylin, the specimen are graded using a microscope. In dry eye states, there is a decrease in goblet cell counts.

Biopsy of the Labial Accessory Salivary Glands: Obtaining a biopsy specimen of the lobules of the accessory salivary gland is a simple procedure that can be performed ii the office. Five to six lobules are obtained from the accessory salivary gland and sent for hematoxylin-eosin staining and histo-pathological evaluation.

The biopsy specimen is graded by a focus score according to the severity of infiltration of the salivary gland. The presence of diffused lymphocytic and plasma cell infiltration of the accessory salivary gland may suggest Sjogren's syndrome. Other conditions that can cause dry-eye syndrome and may show histo-pathological changes in the accessory salivary gland include smyloidosis, hemochromatosis, and sarcoidosis.

Ocular FerningTest: The ocular Ferning test is a simple, inexpensive qualitative test of the conjunctival mucus that is performed by spreading conjunctival scrapings onto a clean glass slide and allowing the glass slide to dry.

Microscopic arborization (ferning) is observed in normal eyes, whereas patients with cicatrizing conjunctivitis such as ocular pemphigoid, Stevens,-Johnson syndrome, trachoma, or alkali burn my show decreased mucus production and none or reduced ferning. Ocular ferning may be affected by the presence of salts.

Test Results: The diagnosis of dry eye syndrome is made from a combination of the clinical history, a suggestive constellation of abnormalities on Schirmer testing, fluorescein staining, and rose bengal staining and, if available, confirmatory laboratory evidence of increased tear osmolarity and decreased reflex lactoferrin level.

The classification of the severity of the disease can be made into mild, moderate and severe for the purpose of individualizing the therapy.

- Mild dry eye syndrome can be defined in patients who have a Schirmer test of less than 10 mm in 5 minutes and less than one quadrant of staining of the cornea.
- Moderate dry eye syndrome can be defined in a Schirmer test of 5 to 10 mm in 5 minutes with punctate staining of more than one quadrant of the corneal epithelium.
- Severe dry eye syndrome can be defined as diffuse punctuate or confluent staining (with fluorescein and rose bengal) of the corneal epithelium, often with filaments and diffuse punctae or confluent staining of the conjunctival epithelium. The Schirmer test in these patients is less than 5 mm in 5 minutes.

1.5. Present Modalities of treatment: Management strategies of Dry eye syndrome include mainly supplementation of tear preferably substitutes containing methylcellulose or carboxy-methylcellulose or identical substances which are viscous in nature**.** Preservatives used in formulations are known to cause dry eyes. All these drugs do not have any effect on basic path physiology and they provide only symptomatic relief**.**The tear-stimulants such as cholinergic drugs increases the tear production from lacrimal gland by stimulating secretions, but not been used in clinical practice. Tear Preservation can be done by occluding the puncta or minimizing evaporation, But is useful as short-term measure to assess the effect of occluding puncta before resorting to permanent measures. All these drugs do not have any effect on basic path physiology and they provide only symptomatic ctions and inflammation. But discontinuation of antibiotics, steroids and all preservative-containing eye drops is mandatory for relief of symptoms and progressively improving the tear film and ocular surface. In view of the above, there is an urgent need to evolve safe and effective management approach to tackle symptoms, infections and underlying pathology

1.6. Challenges and limitations of current modalities of management: A variety of management approaches comprise avoidance of exacerbating factors, tear stimulation and supplementation, increasing tear retention, and eyelid cleansing and treatment of eye inflammation. Principles of management of dry eyes include mainly supplementation and preservation of tear.

- *Tear Substitutes such as* tear replacers are a mainstay of treatment, preferably containing methylcellulose or carboxy-methylcellulose or identical substances are viscous in nature. Poly Viny1 alcohol improves tear retention by forming a film. Preservatives used in formulations are known to cause dry eyes. Therefore formulations without preservatives for single use (refresh) or with a preservative (Polyquad) which do not have any action on cells are to be used. ***All these drugs do not have any effect on basic path physiology and they provide only symptomatic relief.***
- *Tear Preservation* can be done by occluding the puncta or minimizing evaporation, **but is useful as short-term measure** to assess the effect of occluding puncta before resorting to permanent measures.
- Tear Stimulants such *as* Cholinergic drugs increases the tear production from lacrimal gland by stimulating secretions. ***But they have not been used in clinical practice.*** **(Table-3)**

Table-3.: Contemporary management strategies for Dry eye syndrome and Limitations

Approaches	Modalities and Limitations
Tear Substitutes •***Methyl cellulose*** •***Carboxy methyl cellulose*** •***Poly Viny1 alcohol***	•Preservatives used in formulations are known to cause dry eyes. •All these drugs do not have any effect on basic path physiology and they provide only symptomatic relief.
Tear Preservation	•Tear Preservation can be done by occluding the puncta or minimizing evaporation, •But is useful as short-term measure to assess the effect of occluding puncta before resorting to permanent measures
Tear Stimulants	•Tear Stimulants such as Cholinergic drugs increases the tear production from lacrimal gland by stimulating secretions. •Not been used in clinical practice.

1.7. Need for development of appropriate therapeutic Approaches: Owing to the above challenges of different conventional agents, it is at this juncture that the need for drugs/measures that could effectively tackle Dry eye syndrome. A vast number of indigenous drugs coupled with innumerable claims of their varied uses in alleviating wide range of ophthalmic affections calls for scientific validation for their attributes and principles.

Ayurvedic literatures record more than fifty ophthalmic plant drugs and more than forty metals minerals having diversified pharmacological actions on visual system and adnexa of the eye. **(**Srikanth, N., 2000. The Actions and Uses of Indigenous Ophthalmic Drugs, Chaukhamba Sanskrit Prathisthan, Delhi; Srikanth N, Ancient Ocular Therapeutics-An integrated approach., Ayur Medline Vol.I, April, 1999;SrikanthN,Hazra J., A Bird's eye view on single Metal & Mineral Ophthalmic drugs - An Ayurvedic Pharmacological basis, Sachitra Ayurved, July, 1999**)**

1.8. Basis for designing trial intervention and development of topical dosage form: A critical review of classical literature and scientific studies is evident that the combination of ingredients viz. *Yastimadhu* (*Glyeyrrhiza glabra* Linn.) and *Daruharidra* (*Berberis aristata* DC.) certainly play a significant role in restoring the functions of tear film , prevention of ulceration and related checking inflammatory process and contributory to the comprehensive management of Dry eye syndrome .

Chapter-2
Drug Review

Chapter-2: Drug Review

2.1. Rational behind selection of plants for development of eye drops: Classical references and certain clinical studies on medicinal plants viz. Daruharidra (*Berberis aristata* DC.) and Yastimadhu (*Glycyrrhiza glabra* Linn.) endorse the usefulness of these plants in surface inflammatory ocular lesions such as Dry Eye Syndrome, inflammatory ocular conditions.

A study intervention comprising of topical and internal use of Daruharidra (*Berberis aristata* DC.) has shown significant improvement in subjective parameters like Dryness, Redness, Photophobia etc. in Dry eye syndrome. Pharmacological actions such as *caksusya* (conducive to vision), *netrya* (conducive to eye), *netrarujahara* (analgesic ophthalmic action), *sodhahara* (anti-inflammatory action) *netra-kanduhara* (anti allergic action), *vrana-ropana* (wound healing effect) are attributed to these drugs. The response obtained after the clinical study could be well understood with the above pharmacological actions ascribed to various ingredients (N.Srikanth. 2000).

Further Different workers have stated that *Yastimadhu* (*Glycyrrhiza glabra* Linn.) has anti-inflammatory, strengthening and regenerative properties. The anti- inflammatory property is ascribed to cortisone-like substance present in the drug that helps reduction in inflammation. It was noticed that Kemicitine succinate, in spite of its antibacterial property, had not been able to show better results than Yastimadhu (*Glycyrrhiza glabra* Linn.) in the cases studied. Further it has also been observed that Daruharidra (*Berberis aristata* DC.) and Yastimadhu (*Glycyrrhizav glabra* Linn.) appeared to show beneficial effects in removing the acute inflammatory features better than Kemictine succinate.(R.N. Chopra and U.N. Dhur.1958).

In a clinical trial conducted on 32 cases of Allergic Conjunctivitis, glycyrrhetinic acid drops were found to be much efficacious in acute as well as long standing cases. (Database on Medicinal Plants Used in Ayurveda Vol. 3). Considering the above, it is evident that the combination of ingredients viz. Daruharidra (*Berberis aristata* DC.) and Yastimadhu (*Glycyrrhiza glabra* Linn.) certainly play a significant role in restoring

the functions of tear film ,prevention of ulceration and related checking inflammatory process and contributory to the comprehensive management of Dry Eye Syndrome .**(Table-1 andTable-2)**

2.2 Drug profile: The detailed drug profile of Daruharidra (*Berberis aristata* DC.) and Yastimadhu (*Glycyrrhiza glabra* Linn.) portrays various aspects such as botanical source, classification, quality issues including botanical, phytochemical aspects, pharmacological and therapeutic uses recounted in Ayurvedic texts and contemporary sources.

2.2.1.Daruharidra: Daruharidraconsiat of dried stem of *Berberis aristata* DC. (Fam.Berberidaceae); an erect, spinous, deciduous shrub, usually 1.8-3.6 m in height, found in the Himalayan ranges at an elevation of 1000-3000 m, and in the Nilgiri hills in South India.**(Figure-1. and Figure- 2.)**

Taxonomical Classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Ranunculales
Family :	:	Berberidaceae
Genus	:	Berberis
Species	:	aristata

Synonyms

Sanskrit	:	Katamkateri, Darvi
English	:	Indian Berberry
Hindi	:	Daruhaldi, Darhaldi
Bengali	:	Daruharidra
Gujrati	:	Daruharidra, Daruhuladur
Kannada	:	Maradarishana, Maradarishina, Daruhaladi
Malayalam	:	Maramannal, Maramanjal
Marathi	:	Daruhalad
Oriya	:	Daruharidra, Daruhalidi
Punjabi	:	Sumalu
Tamil	:	Gangeti, Varatiumanjal
Telugu	:	Manupasupu
Urdu	:	Darhald
Farsi name	:	Darchoba

Morphology: *Berberis aristata* is a shrub grows up to 6-18 feet in height, with a thick woody root covered with Bark.

Bark: It is thin brittle bark that appears yellow to brown from the outside and deep yellow from the inside. The bark is covered with three-branched thorns, which are modified leaves, and can be removed by hand in longitudinal strips.

Leaves: The leaves are simple, cylindrical, straight, tapering, very sharp, hard, and leathery in texture and are toothed, with several to many small indentations along the margin. These are arranged in tufts of 5-8 and are approximately 4.9 centimeters long and 1.8 centimeters broad. Leaves are deep green on the dorsal surface and light green on the ventral surface.

Flowers: The flowers are yellow, numerous, stalked, hermaphroditic and arranged in drooping racemes. The average diameter of a fully opened flower is 12.5 millimeters. The flowers form a racemose inflorescence, with 11 to 16 flowers per raceme, arranged along a central stem. The flowering season begins in mid-March and lasts throughout the month of April.

Fruit: The plant produces bunches of succulent, acidic, edible berries that are bright aconite violet in color. The berries are ovoid and smooth, approximately 7 millimeters long, 4 millimeters in diameter and weigh about 227 milligrams. The fruits start ripening from the second week of May and continue to do so throughout June. The fruiting season, ends abruptly with the commencement of the rainy season.

Seed: These are 2 to 5 in number and vary in color from yellow to pink

Pharmacognosy

a) Macroscopic: Drug available in pieces of variable length and thickness, bark about 0.4 - 0.8 cm.thick, pale yellowish-brown, soft, closely and rather deeply furrowed, rough, brittle, xylem portion yellow, more or less hard, radiate with xylem rays, pith mostly absent, when present small, yellowish-brown when dried, fracture short in bark region, splintery in xylem; taste, bitter.

b) Microscopic: Stem -Shows rhytidoma with cork consisting of 3-45 rectangular and squarish, yellow coloured, thin-walled cells, arranged radially; sieve elements irregular in shape, thin walled, a few cells containing yellowish-brown contents; phloem fibres arranged in tangential rows, consisting of 1-4 cells, each fibre short thick-walled, spindle-shaped, lignified having wide lumen; half inner portion of rhytidoma traversed by secondary phloem rays; phloem rays run obliquely consisting of radially elongated parenchymatous cells, almost all phloem ray cells having single prismatic crystals of calcium oxalate, a few cells of rhytidoma also contain prismatic crystals of calcium oxalate; stone cells also found scattered in phloem ray cells in groups, rarely single, mostly elongated, a few rounded, arranged radially, some of which contain a single prism of calcium oxalate crystals; secondary phloem, a broad zone, consisting of sieve elements and phloem fibers, traversed by multi seriate phloem rays; sieve elements arranged in tangential bands and tangentially compressed cells alternating with single to five rows of phloem fibers, phloem fibers short, lignified, thick-walled having pointed ends; secondary xylem broad consisting of xylem vessels, tracheids, xylem fibers and traversed by multi seriate xylem rays; xylem vessels numerous, small to medium sized, distributed throughout xylem region in groups or in singles, groups of vessels usually arranged radially; isolated vessels cylindrical with rounded or projected at one or both ends with spiral thickening; xylem fibers numerous, lignified, large, thick-walled with wide lumen, and pointed tips; xylem rays quite distinct, straight, multi-seriate, consisting of radially arranged rectangular cells, each ray 30-53 cells high, 8-12 cells wide, a few ray cells containing brown contents.

Powder : Yellow; shows mostly fragments of cork cells, sieve elements, yellow colored phloem fibers entire or in pieces, stone cells in singles or in groups, numerous prismatic crystals of calcium oxalate, xylem vessels having spiral thickening, thick-walled, lignified xylem fibers and ray cells.

Identity, purity and strength

Foreign matter	-	Not more than 2 per cent
Total Ash	-	Not more than 14 per cent
Acid-insoluble ash	-	Not more than 5 per cent
Alcohol-soluble extractive	-	Not less than 6 per cent
Water-soluble extractive	-	Not less than 8 per cent

Chemical Constituents: Berberine, Oxyberberine, Berbamine, aromoline,karachine, palmatine, Oxycanthine and taxilamine are the main constituents reported from various parts of the plant.(Database on Medicinal Plants Used in Ayurveda Vol. 1)

Ayurvedic pharmacological profile

Rasa	:	Tikta, kashaya
Guna	:	Ruksha, laghu
Virya	:	Ushna
Vipaka	:	katu

Pharmacology: Hypoglycaemic, anti-cancer, gastro-irritant, antifatigue, anticoagulant, antipyretic, local anesthetic, antiprotozoal, anti-TB, anti-bacterial, anti-tumour, hypotensive, anti-inflammatory, anti-trachoma, CNS depressant. Its water extract is called as Rasanjana, used in eye disorders with infection and inflammation. Application of berberine to chronic trachoma patients by intra-conjuctival injection proved highly effective.(Database on Medicinal Plants Used in Ayurveda Vol. 1)

Actions and Uses (Karma): *Netrya* (conducive to eye), *Aksidosahara* (eye diseases), *Kaphabhisyanda-nasana* (ocular inflammation), *vranaropana* (wound-healing),*shophahara* (anti-inflammatory).

Part used: Bark, root, stem, fruit

1.Ayurvedic pharmacopeia of India, Department of AYUSH, Ministry of Health and Family Welfare ,Government of India, Part-I, Vol.II, Pg33-34 First Edition: 1999

2.Database on Medicinal Plants Used in Ayurveda , Central Council for Research in Ayurveda and Siddha , Department of AYUSH, Ministry of Health and Family Welfare ,Government of India, Vol.I, Pp. 120-126; 2000

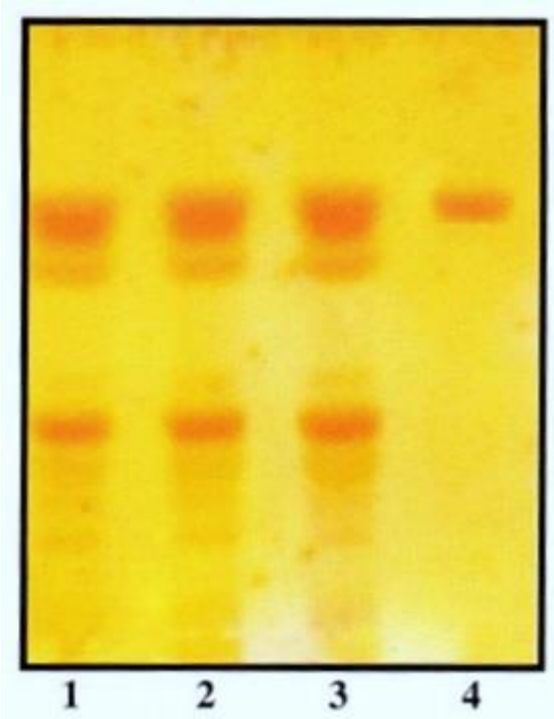
Table-1.Ophthalmic uses and rational behind selection of drug intervention – Daruharidra (*Berberis aristata* DC.)

Drug : Daruharidra(*Berberis aristata* DC.) **Family** :Berberidaceae					
Rasa	Guna	Virya	Vipaka	Pharmacological actions	References
Tikta, Kasaya	Ruksa, Laghu	Usna	Katu	• Netraruja , Kandunasana, Aksidosahara, Kaphabhisyandanasana Netrya, useful in sarvadosaPrakupita,Netrya, Anjananamikahara, Naktandhya-Nasana, Netraroganut[1,] • Anti-bacterial,Anti-histaminic[2] • Sukshakshipaka[3,4,5]	1.N.Srikanth.Actions and uses of Indigenous Ophthalmic drugs(Cross references fromDhanvantaryNighantu, Bhavprakash, Sarangdharasamhita) Pp.15.2. 2.Pharmacological Investigations of certain Medicinal Plant and Compound Formulations used in Ayurveda and Siddha – p. 94-97 3.Sushruta Samhita, Uttarsthana Ch.9/18-22 4.Dhar, M.L. et al. Screening of Indian plants for biological activity Part-I, Indian J. Exptl. Biol. 6: 232, 1968. 5.N.Srikanth. Dry Eye Syndrome and its Management – A clinical study, JRAS, Vol.XXII, and No.1-2 (2001): 17-24.

Fig-1.Daruharidra (*Berberis aristata* DC.)

Plant

Stem



TLC profile of test solution of *Berberis aristata*, stem
1-3 Test solution; 4 Berberine standard

Fig-2.Powder microscopy of *Berberis aristata*.var. *Aristata,* stem

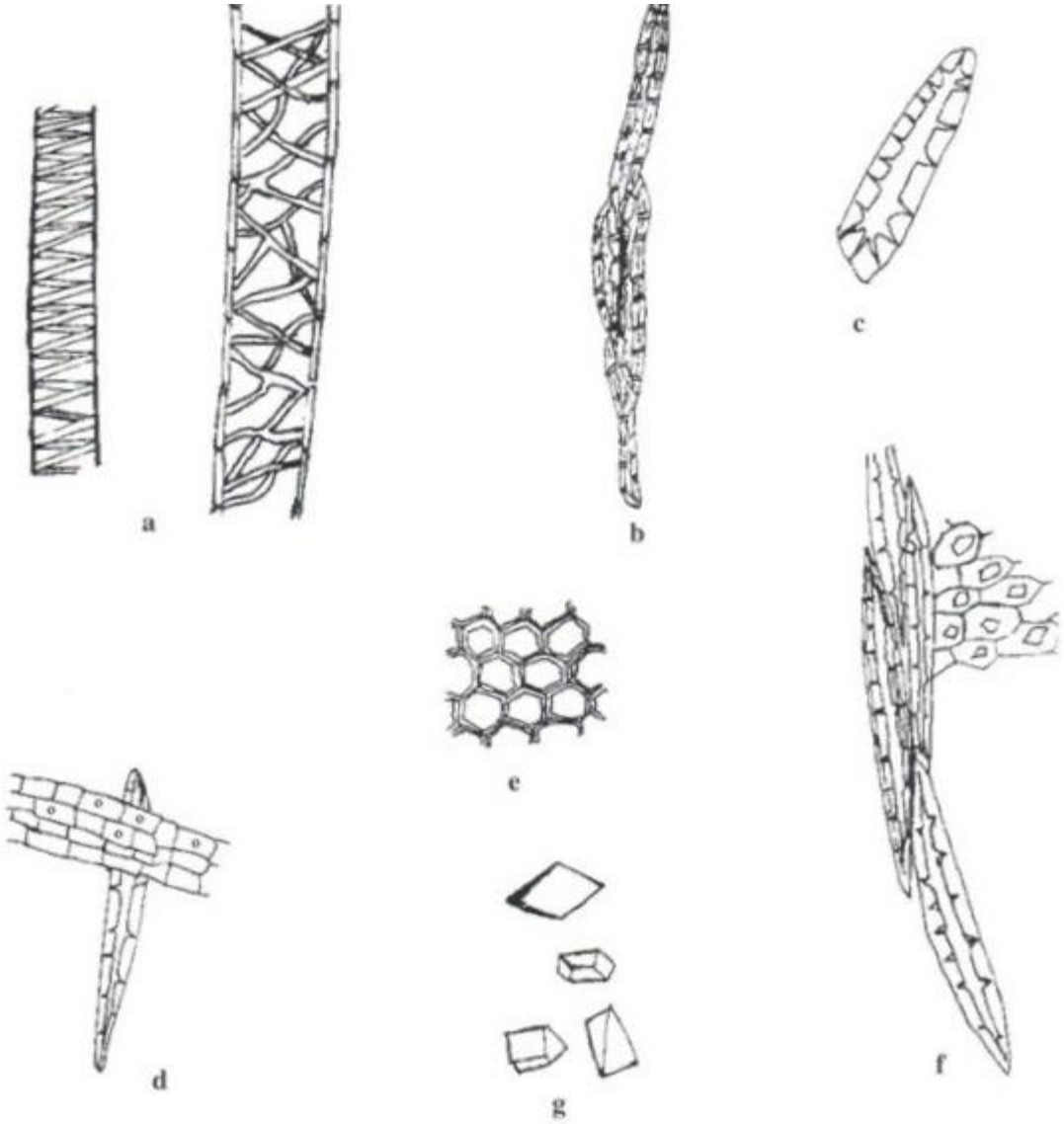


Fig: Powder microscopy of *Berberisaristata*var.*Aristata* stem.**a.** vessels with spiral and reticulate thickening (x 750); **b.**fibresclereids (x 182); **c.** stone cells; **d.**radially cut medullary rays containing starch grains, associated with fibres (x 182); **e.** cork cells in surface view (x 182); **f.** fibers associated with parenchyma (x 182); **g.** prismatic crystals of calcium oxalate.

2.2.2.Yastimadhu: Yastimadhuconsists of dried, unpeeled, stolon and root of *Glycyrrhiza glabra* Linn., Fabaceae, a tall perennial herb, upto 2 meters high, distributed in the sub-tropical and warm temperate regions of the world, mainly cultivated in Europe, Persia, Afghanistan and to little extent in some parts of India. The extreme sweetness of Yasthimadhu, made it a real oddity, due to this, it is used in many medicines to mask the unpleasant taste of the other ingredients. .**(Figure-3. and Figure- 4.)**

Taxonomical Classification

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Super division	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Order	:	Fabales
Family	:	Fabaceae
Genus	:	Glycyrrhiza
Species	:	glabra

Synonyms

Sanskrit	:	Yastimadhuka, Yastika, Madhuka, Madhuyasti, Yastyahva
Assamese	:	Jesthimadhu, Yeshtmadhu
Bengali	:	Yashtimadhu
English	:	Liquorice root
Gujrati	:	Jethimadha, Jethimard, Jethimadh
Hindi	:	Mulethi, Mulathi, Muleti, Jethimadhu, Jethimadh
Kannada	:	estamadu, Madhuka, Jyeshtamadhu, Atimadhura
Kashmiri	:	Multhi
Malayalam	:	Irattimadhuram
Marathi	:	Jesthamadh
Oriya	:	Jatimadhu, Jastimadhu
Punjabi	:	Jethimadh, Mulathi
Tamil	:	Athimadhuram
Telugu	:	Atimadhuramu
Urdu	:	Mulethi, Asl-us-sus

Propagation: The propagation of the plant is done with young pieces of stolons and each piece should exhibit 2-3 buds of aerial shoot. The plant requires a soil 3 to 4 feet deep or more, having a light, loamy and stone-free texture. It is usually grown continuously on the same land. The pieces of stolons are planted in March at 2' by 3' distance. The roots are harvested 3-4 years after planting when they show sufficient growth.

Morphology: It is a perineal herb/subshrub for subtropical and temperate zone. The plant attains a maximum height up to 2m.

Stem: the stem is woody, erect and multi-branched.The underground stem grow horizontally up to 2m length, consisting of short taproot with large number of rhizomes.

Root: The diameter of the root varies from 0.75 to 2.5 cm, grey- brown exterior and yellow interior. Externally, it is longitudinally wrinkled with patches of cork. [15] It has a characteristic pleasant sweet taste.

Leaves: Leaves are alternate, pinnate, yellow green and 10 to 20 cm long. The leaflets are in 3 to 8 pairs and are covered with soft hairs on underside.

Flowers : The axillary inflorescences are upright, spike-like and 10 to 15 cm long. The individual flowers are 1 to 1.5 cm long, bluish to pale violet and short-pedicled

Fruit : The fruit is a pod, 1.5 to 2.5 cm long, and 4 to 6 mm wide. It is erect and splayed, flat with thick sutures, glabrous, somewhat reticulate-pitted, and usually has 3 to 5 brown, reniformseeds.Flowering fruiting is from August to February.

Pharmacognopsy

a) Macroscopic: Stolon consists of yellowish brown or dark brown outer layer, externally longitudinally wrinkled, with occasional small buds and encircling scale leaves, smoothed transversely, cut surface shows a cambium ring about one-third of

radius from outer surface and a small central pith, root similar without a pith, fracture, coarsely fibrous in bark and splintery in wood, odour, faint and characteristic, taste, sweetish.

b) Microscopic: Stolon - Transverse section of stolon shows cork of 10-20 or more layers of tabular cells, outer layers with reddish-brown amorphous contents, inner 3 or 4 rows having thicker, colourless walls, secondary cortex usually of 1-3 layers of radially arranged parenchymatous cells containing isolated prisms of calcium oxalate, secondary phloem abroad band, cells of inner part cellulosic and outer lignified, radially arranged groups of about 10-50 fibres, surrounded by a sheath of parenchyma cells, each usually containing a prism of calcium oxalate about 10-35 μ long, cambium form tissue of 3 or more layers of cells, secondary xylem distinctly radiate with medullary rays, 3-5 cells wide, vessels about 80-200 μ in diameter with thick, yellow, pitted, reticulatelythickend walls, groups of lignified fibres with crystal sheaths similar to those of phloem, xylem parenchyma of two kinds, those between the vessels having thick pitted walls without inter-cellular spaces, the remaining with thin walls, pith of parenchymatous cells in longitudinal rows, with inter-cellular spaces.

Root- Transverse section of root shows structure closely resembling that of stolonexcept that no medulla is present, xylem tetrarch , usually four principal medullary rays at right angles to each other, in peeled drug cork shows phelloderm and sometimes without secondary phloem all parenchymatous tissues containing abundant, simple, oval or rounded starch grains, 2-20 μ in length.

Chemical Constituents:: Glycyrrhizin, glycyrrhizic acid, glycyrrhetinic acid, asparagines, sugars resin and starch.

Identity, purity and strength

Total Ash	:	Not more than 10 per cent
Acid-insoluble ash	:	Not more than 2.5 per cent
Alcohol-soluble extractive	:	Not less than 10 per cent
Water-soluble extractive	:	Not less than 20 per cent

Ayurvedic pharmacological profile

Rasa	:	Madhura
Guna	:	Guru, Snigdha
Virya	:	sita
Vipaka	:	Madhura

Pharmacology: Smoothmuscle depressant, anti-microbial, hypolipidaemic, anti-antherosclerotic, antiviral, hypotensive,hepato-protective,antiulcer,antiuretic, antioxidant,anti-inflammatory, anti-nociceptive, expectorant.

Actions and Uses(Karma): *Caksusyaya* (conducive to eye and vision),*Jeevaneeya* (promote longevity), *Sandhaneeya* (help in bone fracture and wound healing) .

:

1.Ayurvedic Pharmacopeia of India, Department of AYUSH, Ministry of Health and Family Welfare ,Government of India, Part-I, Vol. I, First Edition 1990, reprint, 2001, Pp 127-128,

2. Database on Medicinal Plants Used in Ayurveda , Central Council for Research in Ayurveda and Siddha , Department of AYUSH, Ministry of Health and Family Welfare, Government of India, Vol. III, Pp. 561-671; 2001

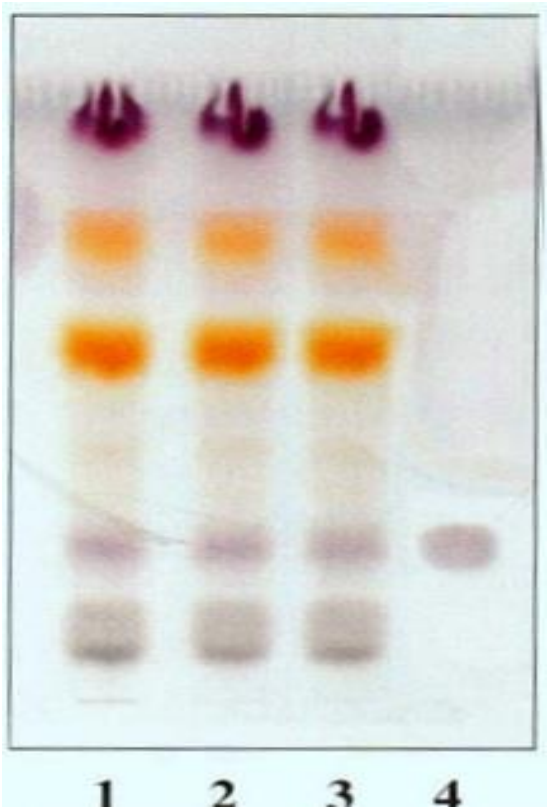
Table-2.Ophthalmic uses and rational behind selection of drug intervention – Yastimadhu (*Glycyrrhiza glabra* Linn.)

Drug : Yastimadhu**(***Glycyrrhiza glabra* Linn) **Family** :Fabaceae					
Rasa	**Guna**	**Virya**	**Vipaka**	**Pharmacological actions**	**References**
Guru Snigdha	Madhura	Ushna	Madhura	Chaksusya, Balya, Timira Hara, VranaRopana [1-5]	1.Actions and uses of Indigenous Ophthalmic drugs (Cross references from Dhanvantari Nighantu, Bhavprakash, Sarangdhar Samhita) Pp. 28 2. J.P.N. Chanssuria, Studies on would healing and effect of indigenous drugs on it. Page N-198,1975 3. R.N. Chopra and U.N. Dhur Indigenous drugs of India U.N. Dhur and Sons Ltd. Calcutta. 1958 4. N.Srikanth,Management of Dry Eye Syndrome- A Case Report Ayur Medline, Vol.IV Jan-June 2001.Pp. 460-463

Fig-3.Yastimadhu (*Glycyrrhiza glabrla* Linn.)

Plant

Root



TLC profile of test solution of *Glycyrrhiza glabrla,* root
1-3 Test solution; 4 Glycyrrhizin standard

Fig-4.Powder microscopy of *Glycyrrhiza glabra*, root and stolon

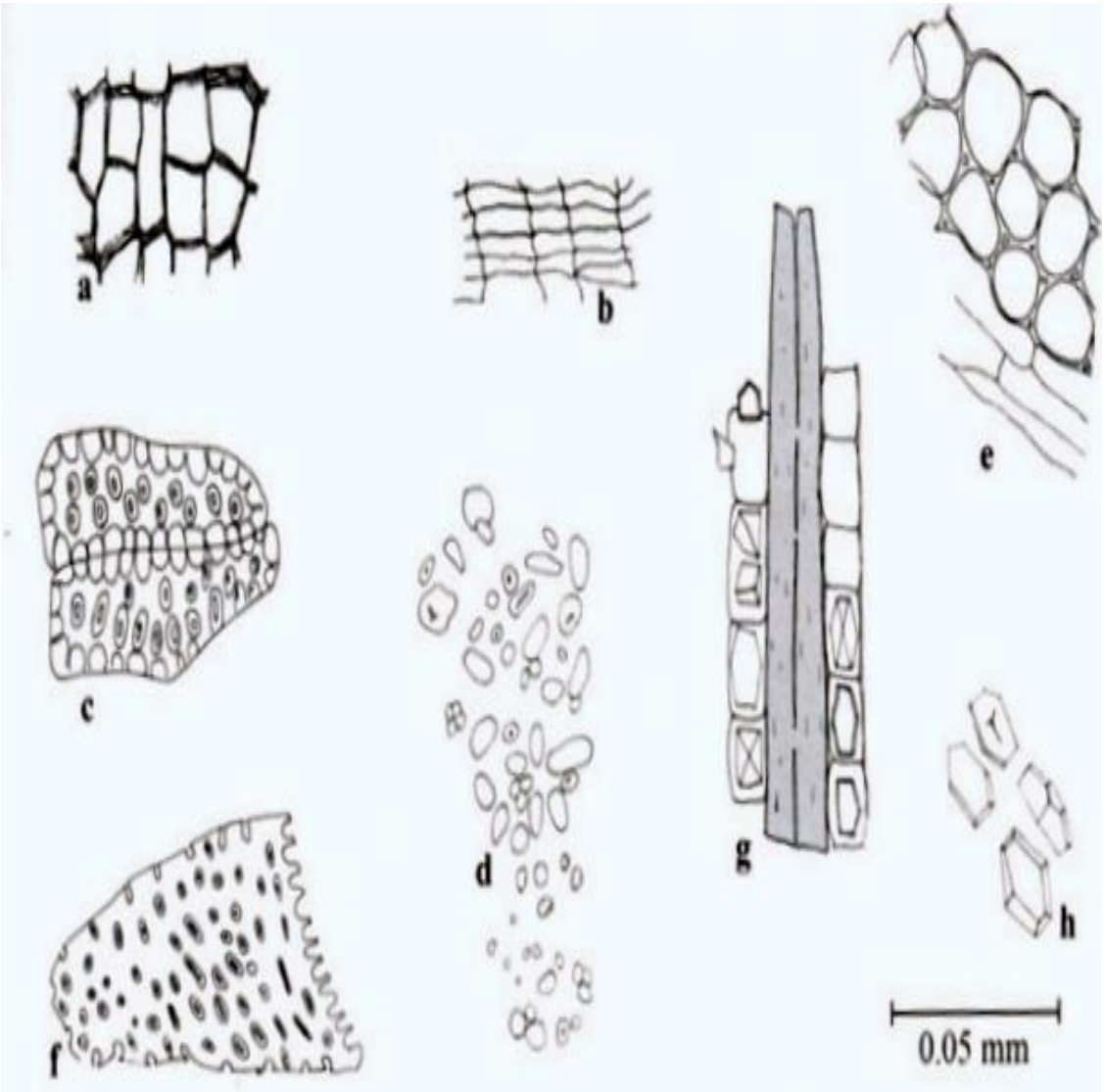


Fig: Powder microscopy of *Glycyrrhiza glabra* root and stolon. **a,** cork in surface vie**b,** transversely cut cork; **c,** group of tracheids showing bordered pits; **d,** starch grains; **e,** tangentially cut medullary ray cells with associated parenchyma; **f,** fragment of vessel with elongated borde pits; **g,** crystal fibers; **h,** prismatic crystals of calcium oxalate.

Classical References

दार्वीक्वाथसमं क्षीर पादंपक्तवा यदा घनम्। तदा रसाञजनंख्यातं नेत्रयो परंम हितम् ।।

(भाव प्रकाश हरीतक्यादिवर्ग /**205)**

तिक्तादारूहरिद्रा स्यात् रूक्षोष्ण व्रणमेहजित।

कर्णनेत्रामुखोद्भूतां रूजं कण्डू च नाशयेत् ।। (धन्वन्तरि निघण्टु गुडूच्यादिवर्ग /**59)**

दार्वीक्वाथसमं क्षीर पादंपक्तवा यदा घनम्। तदा रसाञजनंख्यातं नेत्रयो परंम हितम् ।।

रसाञजनं कटु श्लेष्मविषनेत्रविकारनुत् । उष्णं रसायनं तिक्तं छेदनं व्रणदोषकृत् ।।

(भाव प्रकाश हरीतक्यादिवर्ग /**205)**

यष्टी हिमा गुरूः स्वाद्वी चक्षुष्या बलवर्णकृत ।

सुस्निगधा शुक्ला केश्या स्वर्या पित्तानिलास्रजित ।।

व्रणशोथ विषच्छर्दि तृष्ण ग्लानि क्ष्यापहा ।

(भाव प्रकाश हरीतक्यादिवर्ग /**146)**

Chapter-3
Study outline and Methodology

Chapter-3: Study outline and Methodology

The research plan broadly comprises of pre -clinical and clinical studies involving various steps of drug development to ensure the quality, safety and efficacy. The pre-clinical studies encompass; pharmaceutical development and standardization, ocular safety and toxicity studies, antimicrobial assays and In vitro Biochemical assessment of antioxidant potential of eye drops. The aims and objectives are as under:

3.1. Aim

- Development and validation of safe and effective standardized eye drops for dry eye syndrome (*shushkakshipaka*)

3.2. Objectives

1. To evaluate the clinical efficacy of‟ DY Eye drops' {prepared with *Daruharidra* (*Berberis aristata* DC.) &*Yastimadhu*(*Glyeyrrhizac glabra* Linn.) in Dry Eye Syndrom*e (Shushkakshipak*a)
2. To compare the efficacy of 'DY Eye drops' with Artificial tears (conventional control intervention-Tear supplement- Carboxy methyl cellulose)

3.3. Methodology

1. Pre –Clinical studies: Pre-clinical studies comprise pharmaceutical development and standardization, in vivo ocular safety and toxicity studies, in vitro antimicrobial assays and biochemical assessment of antioxidant potential of eye drops

A.Pharmaceutical development and Standardization: Botanical and chemical standardization , development of standard operational procedures (SoPs) in compliance to Indian Pharmacopoeia. Indian Pharmacopoeia Committee, Ministry of Health and Family Welfare, Government of India, New Delhi, Vol 3, pp1436-1437, 2007. ; Ayurvedic Pharmacopoeia of India, Department of AYUSH, Ministry of Health and Family Welfare, Government of India, Part-I, Vol-I, First Edition,1986; Ayurvedic Pharmacopoeia of India, Department of AYUSH, Ministry of Health and Family Welfare, Government of India, Part-I, Vol-II, First Edition, , Protocol For Testing of ASU Drugs, Pharmacopeial Laboratory for Indian Medicine, Department of AYUSH, Govt. India, 2008.

B. Ocular Safety and Toxicity Studies: Acute eye irritation study in rabbit to assess the toxic characteristics of the eye drops in to rabbit eye in a single dose in full compliance with the guidelines laid down in OECD 405 - OECD GUIDELINE FOR THE TESTING OF CHEMICALS (Acute Eye Irritation/Corrosion) Adopted 24 April 2002.and other prevalent validated and published methods viz..Draise, J.H. The Appraisal of Chemicals in Foods, Drugs, and cosmetics pp. 46-48. Association of Food and Drug Officials of United Statesm, Austin, Texax1959.;Draise, J.H. Appraisal of the Safety of chemicals in Foods, Drugs and Cosmetics; pp 46-59. Association of Food and Drugs official of the United States, Topeka, kanasas 1965. Revised guidelines for research in transgenic plants & Guidelines for toxicity and allergen city evaluation of Transgenic seeds, plants and plant parts, , Department of Biotechnology, Ministry of Science and Technology, Govt. of India ,1999 and Kay JH and Calandra JC), Interpretation of eye irritation tests. *J Sco Cos Chem*; 13:281-289.1962.

C. Antimicrobial Assays of Eye Drops: Antibacterial and antifungal assays of for determining the Minimum Inhibitory Concentration (MIC)using pathogenic strains of bacteria; *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhinurium*, *Escherichia coli*, *Klebsiella pneumonia* as test organisms and screening of antifungal activity of eye drops against *Aspergillus fumigatus and Candida albicans* ,as per prevalent validated and published methods viz. Perumal S, Pillai S, Cai LW, Mahmud R, Ramanathan S. Determination of Minimum Inhibitory Concentration of Euphorbia hirta (L.) extracts by Tetrazolium MicroplateAssay. Journal of Natural Products, 2012, 5: 68-76..

D. *In vitro* Biochemical assessment of antioxidant potential:The study aims at ***In vitro***biochemical antioxidant screening comprising ofNitric oxide radical scavenging assay, **ABTS** radical scavenging assay, DPPH radical scavenging assay and Ferric reducing antioxidant potential (FRAP) assay adopting prevalent validated and published methods viz.Re.R, Pellegrini N, Protoggenete A, Pannala A, Yang M, Rice-Evans C. (1999). Antioxidnat activity applying an improved

ABTS radical cationdecoloration assay. Free RadicBiol Med, 26: 1231-1237;Gomez-Alonso S, Fregapane G, Salvador MD, Gordon MH. (2003). Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. J Agric Food Chem, 51: 667-672.;Blois MS. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181: 1199-1200 and Oyaizu M. (1986). Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition, 44: 307-315.

2. Clinical Study: The clinical study is an interventional randomized open label prospective trial with the study design as under :

Study Type	:	Interventional
Purpose	:	Treatment
Masking	:	Open label
Control	:	Randomized controlled
Timing	:	Prospective
End Point	:	Efficacy
No. of Groups	:	Two
Sample size	:	150(75 participants each in test and control group)

Drug Interventions

Group-I: Installation of 'DY Eye drops' {prepared with *Daruharidra* (*Berberis aristata* DC.) &*Yastimadhu*(*Glyeyrrhiza glabra* Linn.)} three drops for three times a day for one month

Group-II: Installation of 'Artificial tears (conventional Control -Tear supplement- Carboxy methyl cellulose) three drops for three times a day for one month

Chapter- 4
Pre-Clinical Studies

Chapter-4: Pre-Clinical Studies

The pre-clinical studies include various steps involved in drug development which are essential to comply with the requirements of the quality, safety and efficacy viz. pharmaceutical development and standardization, in vivo ocular safety and toxicity studies; *in vitro* antimicrobial assays and biochemical assessment of antioxidant potential of eye drops which are contributory to inclusive approach in the management of dry eye syndrome.

4.1. Pharmaceutical development and Standardization

1. Raw drug identification and quality assurance: Raw ingredients viz. dried unpeeled stolon and root of *Yastimadhu* (*Glycyrrhiza glabrla* Linn.) and dried stem of *Daruharidra* (*Berberis aristata* DC.) procured from authentic market sources**(Fig.-1).** The identity was confirmed with compliance of microscopic, macroscopic parameters of Ayurvedic pharmacopoeia of India (API) through pharmacognosy studies. The purity and strength were also confirmed through physico-chemical studies done as per 'Protocol For Testing of ASU Drugs, Pharmacopeial Laboratory for Indian Medicine, Ministry of AYUSH, Govt. India and compliant with parameters of Ayurvedic pharmacopoeia of India(API) (**Table-1 and Table-2)**

Fig.1-Samples of ingredients of Eye Drops

***Daruharidra* (*Berberis aristata* DC.)**

***Yastimadhu* (*Glycyrrhiza glabrla* Linn.)**

Table-1.Physico-chemical studies of *Yastimadhu* (*Glycyrrhiza glabrla* Linn.)

SNo.	Parameters	Results
1.	Total ash	7.92
2.	Acid –insoluble ash	0.62
3.	Water soluble extractive	25.69
4.	Alcohol soluble extractive	23.37
5.	pH (1% w/v aqueous solution)	5.52

Table-2.Physico-chemical studies of *Daruharidra* (*Berberis aristata* DC.)

SNo.	Parameters	Results
1.	Loss on drying at 105°C (% w/w)	5.13
2.	Total ash	2.10
3.	Acid-insoluble ash (% w/w)	0.01
4.	Water-soluble extractive (% w/w)	10.64
5.	Alcohol soluble extractive (% w/w)	6.45

2.Standard Operative Procedures (SoPs) for eye drops development and analytical specifications: The step wise development of eye drops encompass the preparation of distillate, making of the distillate isotonic to lacrimal fluid and adjustment of pH, addition of preservative and packing under sterile conditions. 50g powder (particles passed through 40-mesh) of each of dried unpeeled stolon and root of *Yastimadhu* (*Glycyrrhiza glabrla* Linn.) and dried stem of *Daruharidra* (*Berberis aristata* DC.) of pharmacopeia quality was soaked in 850 ml of distilled water for overnight in an air tight container. The material was transferred to a distillation unit. Distillate was obtained by adjusting the temperature to 40^{o}C for 15 minutes and raising the temperature slowly to 80^{o} C. The first 450 ml. of distillate was collected at the rate of 20 drops per minutes in anairtight

container. The distillate was made isotonic to lacrimal fluid by adding 0.9% NaCl to distillate and dissolving properly and adding isotonic phosphate buffer viz. 0.16 g. of monobasic Sodium phosphate and 0.76g of dibasic Sodium phosphate. Finally the pH of the eye drops was adjusted to 6.9-7.30.

Benzalkonium chloride in a ratio of 1:10000, was added as preservative and pH was again checked and found within the specified range of the ophthalmic drops (pH 6.9-7.30). Test for sterility performed after addition of preservative the preparation was observed for 48hours, and found sterile. The packing was made in autoclaved sterilized amber glass containers of10 ml. Capacity**.** The finished product tested for quality assurance and safety and the analytical specifications complied specified parameters of Indian pharmacopeia for ophthalmic preparations (**Table-3).**

Table-3. Analytical Specifications of Eye Drops

Descriptions	Colorless clear solution with mild characteristic odor
Particulate matter	Passes as per Indian Pharmacopeia, 1996. (when examined under suitable conditions of visibility, are clear and practically free from particles that can be observed on visual inspection by unaided eye.)
Identification	Passes Liberman Burchard test Extract 10 ml. eye drops in vol. of extract to one ml. add one drop of acetic anhydride and keep it for a drop of conc. Sulphuric acid from the side of test tube a violet color ring appears at the junction of two liquids which gradually disappears.
pH	6.9-7.3
Sodium chloride content(%)	0.85-0.95% w/v
Sterility test	Passes (Indian Pharmacopeia,1996)

3. Quality standards of herbal eye drops: The thin layer chromatographic (TLC) profiling of raw ingredients of *Yastimadhu* (*Glycyrrhiza glabrla*Linn.) *Daruharidra* (*Berberis aristata* DC.) was done in the following way. 1.0g each of *these* ingredients were soaked overnight separately in 10 ml of 70% methanol.

The solutions were continuously stirred for 6 hrs and kept for next 18 hrs. Next day filtered the samples, dried and Made 10% solutions. Later 50ml of sample(eye drop), was concentrated to volume of 1.0ml over waterbath maintained at 80-90^{o}C & add 1.0ml of methanol. 5µl each of these solutions were applied separately in 10mm band length on Merck Aluminum plate pre-coated with silica gel 60 F_{254} of 0.2mm thickness with the help of Linomat IV applicator.

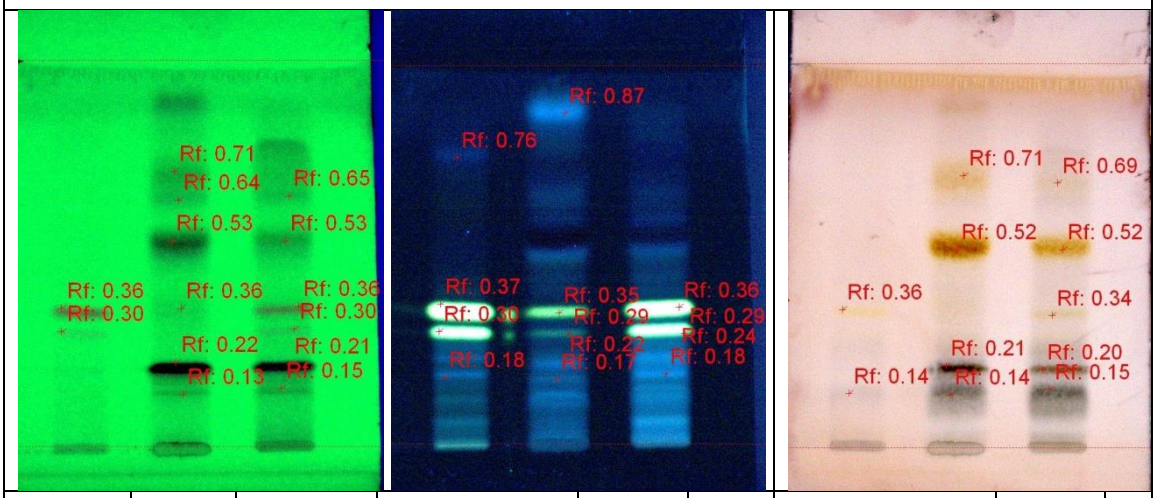
The plate was developed in Twin trough glass chamberusing mobile phase n-Butanol: Water: Acetic Acid (7:2:1). The plate was dried in air and visualized under λ 254 nm and λ 366 nm for ultra violet detection and taken the fingerprints.

Later the plate was dipped in Anisaldehyde – Sulphuric acid and heated at 105°C till the colour of the spots appeared and fingerprint taken under white light. The TLC profiling of finished product of eye drops was matched with that of ingredients (**Table-4) & (Fig-2).**

Thin Layer Chromatographic (TLC) Profiles of ingredients and finished product

Table-4: Observations of Thin Layer Chromatographic (TLC) Profiles of ingredients and finished product			
Sample	**Visualization/ Detection (R_f Values)**		
	Under UV 254nm	**Under UV 366nm**	**Derivatized**
Berberis aristata DC.	0.30, 0.36	0.18, 0.30, 0.37, 0.76	0.14, 0.36
Glycyrrhiz aglabra Linn.	0.13, 0.22, 0.36, 0.53, 0.64, 0.71	0.17, 0.22, 0.29, 0.35, 0.87	0.14, 0.21, 0.52, 0.71
Eye drop(finished product)	0.15, 0.21, 0.30, 0.36, 0.53, 0.65	0.18, 0.24, 0.29, 0.36	0.15, 0.20, 0.34, 0.52, 0.69

Fig-2. Thin Layer Chromatographic (TLC) Profiles of ingredients and finished product



A	B	C	A	B	C	A	B	C
254nm			366nm			Derivatized		

A-*Berberis aristata* DC., *B-Glycyrrhiza glabra* Linn., C- Eye drop (*formulation)*

Quality standards of Indian Medicinal Plants, Indian Council of Medical Research, Vol. 9, 2011, pp 175-186.(*Glycyrrhiza glabra*).

Quality standards of Indian Medicinal Plants, Indian Council of Medical Research, Vol. 3, 2005, pp 78-87.(*Berberis aristata*).

4.2. Ocular Safety and Toxicity Studies: The objective of this Acute eye irritation study in rabbit was to assess the toxic characteristics of the eye when instilled in to rabbit eye in a single dose. The study conducted in full compliance with the guidelines laid down in OECD 405- Guidelines for the testing of chemicals (Acute Eye Irritation/Corrosion) as adopted on 24 April 2002 and other prevalent guidelines

1. Test System and methodology

Test system	:	Rabbit
Strain	:	New Zealand White
Age	:	12 to 14 weeks
Body weight range at Initiation	:	1.925 kg to 2.180 kg
Identification	:	By cage tag and corresponding colour body markings
Number & sex of animals per step per group	:	Six (3 males +3 females)
Acclimatization	:	One week in experimental room after veterinary examination
Randomization	:	After acclimatization and veterinary examination animals were randomly selected.
Nutritional conditions	:	Fasted four hours prior to treatment. Food was offered one hour after dosing.
Husbandry		
Environmental conditions	:	Air conditioned rooms with 10 - 15 air changes per hour, temperature between 21±2 0C, relative humidity 55 ±5 % and illumination cycle set to 12 hours artificial fluorescent light and 12 hours dark.=
Accommodation	:	Individually housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
Diet	:	Nutrimix' brand pelleted standard rabbit feed Manufactured by Nutrivet Life Sciences, Panchal Nivas, Uruli, Devachi Fata, Saswad Road, Pune, was provided ad libitum.
Water	:	Potable water passed through reverse osmosis filtration system and exposed to UV ray was provided ad libitum in glass bottles with stainless steel sipper tubes.

2. Preparation of Animals: Both eyes of each experimental animal provisionally selected for testing were examined within 24 hours before testing starts. Animals showing eye irritation, ocular defects, or pre-existing corneal injury will not be used

3. Study Design: After an Acclimatization period and pre examination the rabbits were weighed and the required numbers of animals were randomly allocated to the treatment group. As described below, this group of rabbits was instilled eye drops (0.1 ml) into the right eye and was observed for the eye irritation and clinical sign for 72 hours. The untreated eye serves as the control.

Group No.	Dose (ml)	Female Rabbits	
		Numbers	ID
1	0.1	6	184/RB001 – 184/RB006

4.Administration: One day prior to treatment, the eyes of all the rabbits were examined. The DE DROPS (0.1 ml) was into the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The other eye, which remains untreated, serves as a control.

5.Mortality: On the day of administration, all animals were observed for mortality at 30 min, 1, 2, 4 and 6 hours following topical application and thereafter they were observed once a day for 72 hours.

6. Clinical Signs and Grading of Reactions: The treated animals were observed for signs of intoxication, at 10, 30 min, 1, 2, 4 and 6 hours after application and thereafter once a day for 72 hours. The appearance, progress and disappearance of the signs if any were recorded. Changes, if any, in gait, posture and responses to handling as well as the presence of clonic or tonic movements, stereotypies or bizarre behavior were also recorded.

The eyes were examined at 1, 24, 48, and 72 hours after test substance application. The grades of ocular reaction (conjunctivae, cornea and iris) were recorded at each examination (Table 1). Examinations of reactions were facilitated by use of a binocular loupe, hand ophthalmoscope **(Table-1 to Table-5)**

7. Body weights: The body weights of rabbits were individually recorded before application of eye drops

Table 1.Individual Animal Ophthalmoscopy report

Dose: 0.1 ml/Rabbit Group: G1 Sex: M / F
Study day: 0

ANIMAL ID	184/RB001	184/RB002	184/RB003	184/RB004	184/RB005	184/RB006
Area examined						
Retinal Reflex	N	N	N	N	N	N
[Lids ADNEXA [Ducts [Cornea	N	N	N	N	N	N
Iris	N	N	N	N	N	N
Aqueous Humour	N	N	N	N	N	N
Lens	N	N	N	N	N	N
Vitreous Humour	N	N	N	N	N	N
Retina [Vessels [Macula	N	N	N	N	N	N
Optic Disc	N	N	N	N	N	N
TapetumLucidum	N	N	N	N	N	N
TapetumNigrum	N	N	N	N	N	N

Table- 2.Grading of Ocular lesions

A .Cornea
Opacity: degree of density (readings should be taken from most dense area)*
No ulceration or opacity..... 0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible 1
Easily discernible translucent area; details of iris slightly obscured 2
Nacrous area; no details of iris visible; size of pupil barely discernible 3
Opaque cornea; iris not discernible through the opacity 4
Maximum possible: 4
* The area of corneal opacity should be noted
B. Iris
Normal …… ...0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia;
or injection; iris reactive to light (a sluggish reaction is considered to be an effect 1
Hemorrhage, gross destruction, or no reaction to light 2
Maximum possible: …… ……2
Conjunctivae
Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)
Normal ..... ……...0
Some blood vessels hyperaemic (injected) 1
Diffuse, crimson colour; individual vessels not easily discernible 2
Diffuse beefy 3
Maximum possible : 3
D.Chemosis
Swelling (refers to lids and/or nictating membranes)
Normal 0
Some swelling above norma 1
Obvious swelling, with partial eversion of ..... … ..2
Swelling, with lids about half closed ..... ……..3
Swelling, with lids more than half closed ..... ….4
Maximum possible : 4

Table-3.Reference Values for Eye Irritation*

MMTS	Irritation Classification	Requirement for maintenance of classification
0.0 – 0.5	Non	Up to 0.5 at 1 hour with zeros at 24 hours; otherwise, increase one level
0.6 – 2.5	Partially Non	With zeros at 24 hours; otherwise, increase one level
2.6 – 15.0	Minimally	With zeros at 48 hours; otherwise, increase one level
15.1 – 25.0	Mildly	With zeros at 96 hours; otherwise, increase one level
25.1 – 50.0	Moderately	With 7 day mean ≤ 20 and individual total scores ≤ 10 in at least 60% of the rabbits with no total score > 30; otherwise, increase one level
50.1 – 80.0	Severely	With 7 day mean ≤ 40 and individual total scores ≤ 30 in at least 60% of the rabbits with no total score > 60; otherwise, increase one level.
80.1 – 100.0	Extremely	With 7 day mean ≤ 80 and individual total scores ≤ 60 in at least 60% of the rabbits with no total score > 100; otherwise, increase one level.
100.1 - 110	Maximally	With 7 day mean > 80 and individual total scores >60 in at least 60% of the rabbits; otherwise, decrease one level.

**Kay JH and Calandra JC), Interpretation of eye irritation tests. J Sco Cos Chem; 13:281-289. . 1962*

Table 4. Individual Scores for Ocular Irritation

	Scoring (at hours)											
Animal ID	**184/RB001**				**184/RB002**				**184/RB003**			
	1	**24**	**48**	**72**	**1**	**24**	**48**	**72**	**1**	**24**	**48**	**72**
I Cornea												
A. Opacity	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
B. Area	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
(AX B) X 5	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
II Iris												
A. Values	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
A X 5	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
III Conjunctivae												
A. Redness	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
B. Chemosis	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
C. Discharge	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
(A+B+C) X 2	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Total score	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Average score	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
MMTS	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

MMTS= Maximum Mean Total score

Cornea (AXB)X5= Total max. 80, Iris AX5= Total max. 10,
Conjunctivae(A+B+C)X 2= Total max. 20

Table.- 4 (Continued)

Individual Scores for Ocular Irritation

	Scoring (at hours)											
Animal ID	**184/RB004**				**184/RB005**				**184/RB006**			
	1	**24**	**48**	**72**	**1**	**24**	**48**	**72**	**1**	**24**	**48**	**72**
I Cornea												
A. Opacity	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
B. Area.	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
(AX B) X 5	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
II Iris												
A. Vaslues	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
A X 5	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
III Conjunctivae												
A. Redness	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
B. Chemosis	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
C. Discharge	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
(A+B+C) X 2	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Total score	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Average score	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
MMTS	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

MMTS= **Maximum Mean Total score**

Cornea (AXB) X5= Total max. 80, Iris AX5= Total max. 10,
Conjunctivae(A+B+C) X 2= Total max. 20

Table- 5. Irritation Index

Total Number of Rabbits		**Hours**			
		1	**24**	**48**	**72**
6	**MMTS**	**0**	0	0	**0**
	Maximum Average Irritation Index	**0**	0	0	**0**

The incidence, severity and reversibility of irritation data

Time post Instillation	Incidence of Irritation		
	Corneal opacity	Iritis	Conjunctivitis
1 Hour	0/6	0/6	0/6
24 Hours	0/6	0/6	0/6
48 Hours	0/6	0/6	0/6
72 Hours	0/6	0/6	0/6

Time post Instillation	Severity of Irritation Mean score
1 Hour	0
24 Hours	0
48 Hours	0
72 Hours	0

7. Observations and Results

Changes in Ocular mucous membrane: 0.1 ml of the drops was placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The other eye, which remains untreated, serves as a control. The eye drops did not cause irritation to mucous membrane of eyes of rabbits and no evident signs of toxicity were observed. The eye drops in New Zealand White rabbits was found to be nonirritant to the ocular mucous membrane ***(*Table-1 to Table-5)**

Clinical Signs and Mortality: Eye Drops tested (0.1ml), for Acute Eye Irritation test did not cause any mortality and no evident sings of toxicity observed, during the observation period of 72 hours post application.

Conclusion : The Eye drops did not cause irritation to ocular mucous membrane of eyes of rabbits such as opacity ulceration of cornea; congestion, swelling, moderate circumcorneal hyperaemia or injection, hemorrhage, gross destruction of iris; redness and other signs of inflammation of palpebral and bulbar conjunctivae; chemosis of lids etc.and no evident signs of toxicity were observed.

Further, no clinical signs of mortality and change in body weight were noticed during the observation period of 72 hours post application and the eye drops was found to be practically nonirritant to the eyes of rabbits.

4.3. *In vitro* Anti-Microbial assays

1. Management of infection-A Pivotal aspect in Dry Eye Disease: Dry eye disease commonly occurs after an episode of viral kerato-conjunctivitis or severe acute or sub-acute conjunctivitis. These diseases may lead to loss of goblet cells from the conjunctival epithelium and release of inflammatory cytokines. Patients usually complain of persistent symptoms and continue to be treated for the original condition. This treatment is not only inappropriate, it may also be toxic; whereas they are actually suffering from the vicious cycle of secondary tear film alterations

Further, Blepharitis, an extremely frequent cause of dry eye disease, has infectious and inflammatory components It results in impairment of the lipid phase of the tear film and an increased rate of tear evaporation. In addition, dysfunction of the meibomian glands and poor elimination of abnormally thick and viscous lipid secretions provide favorable conditions for secondary bacterial infection at the base of the eyelashes. The toxins released by the bacteria aggravate the condition and produce lesions on the cornea adjacent to the lid margins.

Topical antibiotics and corticosteroids are sometimes used to treat secondary infections and inflammation. But discontinuation of antibiotics, steroids and all preservative-containing eye drops is mandatory for relief of symptoms and progressively improving the tear film and ocular surface. In view of the above, there is an urgent need to evolve safe and effective inclusive management strategy addressing the issues related to infections in dry eye disease.

2. Objective: It is imperative to explore the anti-microbial potential of eye drop, as the control of infection plays a crucial role in dry eye syndrome besides the management of symptoms due to deficient tear film components. The objectives of this study comprise antibacterial and antifungal assays of eye drop formulated for dry eye syndrome. The assay was carried out for determining the Minimum Inhibitory Concentration (MIC) (Perumal S, Pillai S, Cai LW, Mahmud R, Ramanathan S. Determination of Minimum Inhibitory Concentration of Euphorbia hirta(L.) extracts by Tetrazolium Microplate Assay. Journal of Natural Products, 2012, 5: 68-76.)

3. Methodology

A. Antibacterial Assays for Determination of Minimum Inhibitory Concentration (MIC): Tetrazolium Microplate Microbial viability Assay, a colorimetric assay based on the reduction of a tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) for rapidly determining the susceptibility of pathogenic strains to bactericidal Ayurvedic drugs was carried out as described by Perumal*et.al.* Pathogenic strains of bacteria; *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhinurium*, *Escherichia coli*, *Klebsiella pneumonia* were used as test organisms. The eye drops were tested for their antibacterial potential, and results were noted down. The 200 µl of eye drop was mixed with 100 µl bacterial culture in nutrient broth and then inoculated in the 96 well plates. Serial Dilutions were performed according to the protocol and kept for incubation at 37°C for overnight. The drug-free controls (i.e. only bacterial strain) and appropriate blanks (i.e. only eye drops) were included as negative control. After the overnight incubation, cold 20% Tetrazolium solution was added to each well. The colour change was observed and noted for determining the MIC value of respective drug against the bacterial cultures. The bacterial growth was corresponded with the colour change to pink from the original colour of the respective drug and in absence of growth the colour remained the same. The MIC value was determined by observing the pink colour that indicates bacterial growth (+) and colorlessness that indicates inhibition of bacterial growth (-). The minimum concentration of the drug corresponded to the growth inhibition was treated as the 'MIC'.

B. Antifungal assay: Screening of antifungal activity of eye drops, was carried out against *Aspergillus*sp., *Aspergillus fumigatus and Candida albicans* obtained from Microbial Culture Collection, National Centre for Cell Science, Pune, (NCCS). The drug and the fungal culture in the Sabouraud dextrose broth were mixed in the 96 well plate. Dilutions were performed according to the protocol and kept for incubation at 37°C for 3 days. Each day the fungal growth was observed to determine the MIC value of respective drug against three fungal pathogenic strains **.**The MIC value was determined by observing the fungal plaques that indicated growth (+) and their absence indicated no growth (-).

4. Observations and Results

A. Observations of Antibacterial Assays: The MIC values of the various concentrations of eye drops against different strains of bacteria were assessed. After overnight incubation, cold 20% tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5- phenyltetrazolium chloride (INT) was added and changes in colour were recorded**.** Eye drops have demonstrated antibacterial activities against all 5 bacterial strains under study; except *Bacillus subtilis*, the best effect was against *E coli***(Table.1) (Fig.1)**

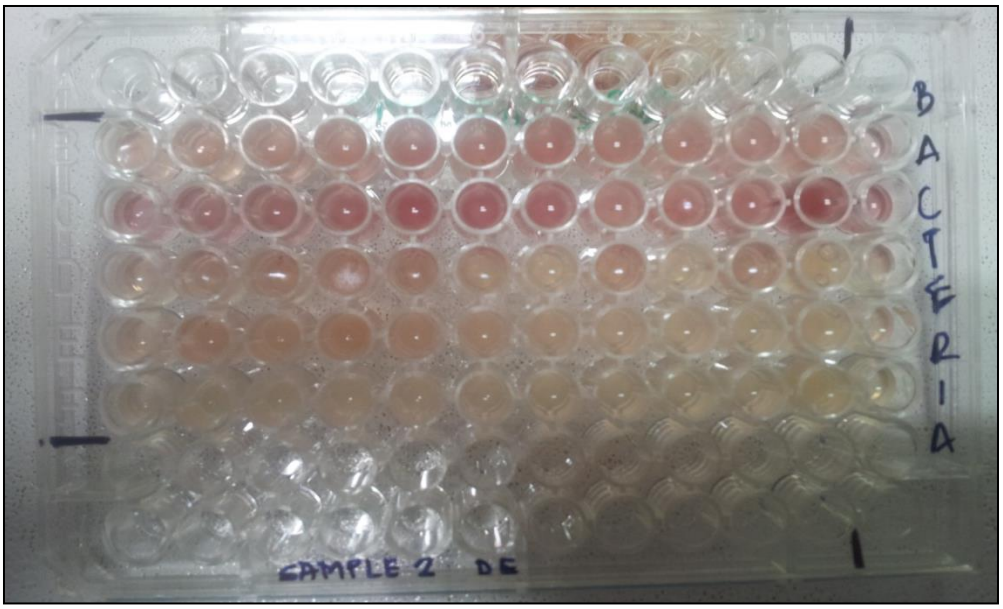
Table 1: Layout and observations made in antimicrobial assay carried out on 96 microwelltitre plate.

Concentration Eye Drop (µg/ml) →		120.0 µg/ml	60.0 µg/ml	30.0 µg/ml	15.0 µg/ml	7.50 µg/ml	3.75 µg/ml	1.87 µg/ml	0.93 µg/ml	0.46 µg/ml	0.23 µg/ml	0.11 µg/ml	Positive Control
BLANK	A												
Staphylococcus aureus	B	-	-	-	+	+	+	+	+	+	+	+	+
Bacillus subtilis	C	+	+	+	+	+	+	+	+	+	+	+	+
Pseudomonas aeruginosa	D	-	-	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumonia	E	-	-	-	+	+	+	+	+	+	+	+	+
Escherichia coli	F	-	-	-	-	-	+	+	+	+	+	+	+
Negative control	G	-	-	-	-	-	-	-	-	-	-	-	+
BLANK	H												

(+): Growth of organisms; (-): No growth of organisms

Antibiotic standard	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Klebsiellapneumonae*	*Bacillus subtilis*
Tetracycline (µg/ml)	10.0	1.25	10.0	0.312	1.25

Figure 1: Observations on colour changes after overnight incubation and adding cold 20% tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5- phenyltetrazolium chloride (INT).



B. Observations of Antifungal assay: The MIC values of the various concentrations of eye drops against different strains screening of antifungal activity of eye drops against *Aspergillus spp., Aspergillus fumigatus and Candida albicans* was done. The eye drops at 200 µl volume has antifungal activities against all 3 fungal strains under study**(Table.2) (Fig.2)**

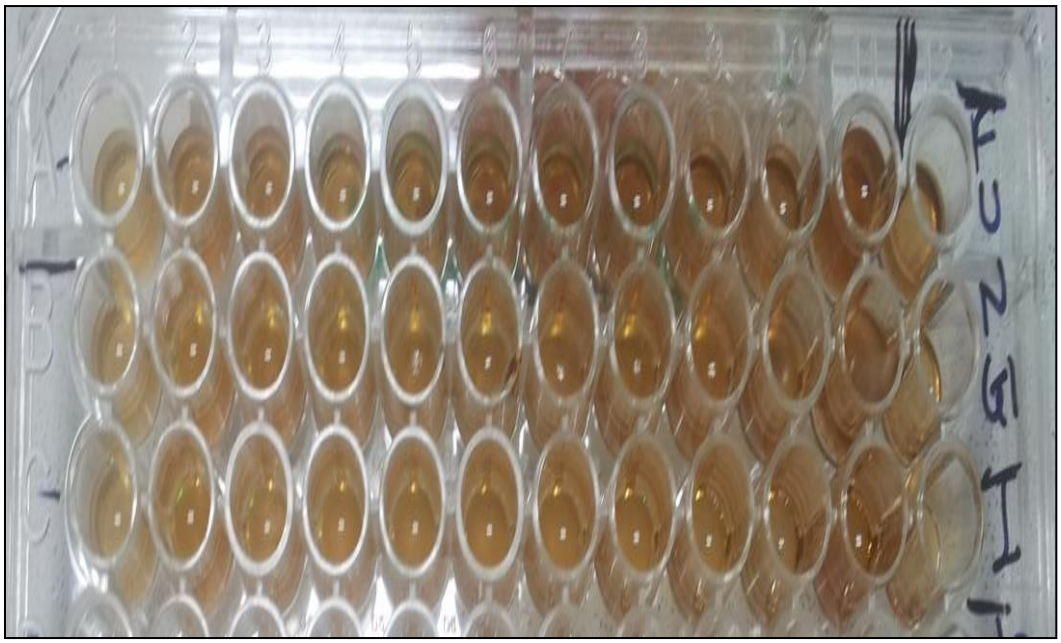
Table 2: Layout and observations made in antimicrobial assay carried out 96 microwelltitre plate.

Concentration Eye Drop (µg/ml)→		120.0 µg/ml	60.0 µg/ml	30.0 µg/ml	15.0 µg/ml	7.50 µg/ml	3.75 µg/ml	1.87 µg/ml	0.93 µg/ml	0.46 µg/ml	0.23 µg/ml	0.11 µg/ml	Positive Control
Aspergillus spp.	A	-	+	+	+	+	+	+	+	+	+	+	+
Aspergillusfumigatus	B	-	+	+	+	+	+	+	+	+	+	+	+
Candida albicans	C	-	+	+	+	+	+	+	+	+	+	+	+

(+): Growth of organisms; (-): No growth of organisms.

Standard Antifungal	**Minimum Inhibitory Concentration (MIC) in µg/ml**		
Amphotericin B	*Aspergillus spp.*	*Aspergillusfumigatus*	*Candida albicans*
	0.156	0.156	0.156

Figure 2: Observations on changes in the turbidity after 72 hrs. incubation



5. Conclusion: In addition to the management of symptoms related to deficiency of tear components, the control of infection also forms an essential constituent in dry eye disease. The close relationship between ocular surface epithelia and the preocular tear film ensures ocular surface health. Dysfunctional protective elements that can lead to ocular surface and tear disorders are heterogeneous, and these different disorders themselves can manifest dysfunctional protective elements. Effective therapeutic strategies need be formulated to manage ocular surface and tear disorders presenting with diverse etiology. The eye drops formulated rationally with Ayurvedic plant ingredients have demonstrated notable anti- microbial activity contributory to the management of dry eye syndrome.

4.4. *In vitro* antimicrobial assays and biochemical assessment of Antioxidant potential

1. Role of antioxidant supplementation in the maintenance ocular surface health and need for assessment of anti-oxidant potential : Oxidative stress in the cornea influenced by several environmental factors such as air pollution, radiation, chemicals etc. leads to changes in corneal optical properties and decrease in visual acuity or even vision loss. The antioxidant agents help in suppressing the damages due to oxidative stress hand assist in restoring the corneal health **(**Cestmir Cejka and Jitka Cejkova. Oxidative Stress to the Cornea, Changes in Corneal Optical Properties, and Advances in Treatment of Corneal Oxidative Injuries. Oxidative Medicine and Cellular Longevity, Volume 2015,**).**

The surface lesions and corneal diseases, associated with oxidative stress leads to corneal aging, b corneal inflammation In acute corneal inflammation the Reactive oxygen species (ROS) are highly involved. Studies on the oxidative reactions in tears of patients with dry eye disease confirmed a marked increase of inflammatory activity in the tear film of patients suffering from dry eye. These reactions lead to severe damage of the eye. Free radicals and inflammation may be involved in the pathogenesis or in the self-propagation of the dry eye disease. The antioxidant therapy with superoxide dismutase and dimethylthiourea are employed for the healing of corneal ulcers evoked by sodium hydroxide. Topical antioxidant therapy found effective in reducing the inflammatory corneal reaction.

Further, the Antioxidant supplements such as vitamin C and vitamin E, probably have an important role in reducing the oxidative damage produced by nitric oxide and other free radicals and improving the ocular surface milieu**.** The medicinal plant ingredients rationally chosen in formulating the eye drops for dry eye disease viz. *Yastimadhu* (*Glycyrrhiza glabrla*Linn.) *Daruharidra* (*Berberis aristata* DC.) possess antioxidant, antimicrobial activity backed by scientific evidence. Methanolic extract of *Berberis aristata* DC has shown DPPH free radical Scavenging Activity expressed in % inhibition with L Ascorbic acid as standard showing IC50 9.6µg/ml

and that of extract was 33.31µg/ml. Hydrogen peroxide radical scavenging activity was comparable to standard IC50 for L Ascorbic acid is 54.23µg/ml and that of *B. aristita* is 60.6µg/ml. Similarly, reducing power of plant extract at different concentration was comparable with L-Ascorbic Acid. The Antimicrobial screening revealed activity against *Candida albicans, Salmonella typhii, Pseudomonas aeruginosaand Escherichia coli***(**Study of phytochemical, antioxidant, antimicrobial and anticancer activityof *Berberis aristita***.(**Basanta Lamichhane, Sandeep Adhikari, Pritish Shrestha and Bhupal Govinda Shrestha.The Journal of Tropical Life Science, Vol.. 4, NO. 1, pp. 01-07 , January, 2014,**)**

In an experimental study, the antioxidant assay of methanaloic extract of *Glycyrrhiza glabra* confirmed the potent antioxidant activity**(**Shapna Sultana. Afroza Haque, Kaiser Hamid, Kaniz Fatima Urmiand Sumon Roy. Antimicrobial, cytotoxic and antioxidant activity of methanolic extract of *Glycyrrhiza glabra.* .AGRICULTURE AND BIOLOGY JOURNAL OF NORTH AMERICA, Agric. Biol. J. N. Am., 2010, 1(5): 957-960)

2. Objective: Reactive oxygen species (ROS) are highly involved in the pathogenesis of dry eye disease and topical and oral antioxidant therapy has shown a protective role in preventing the damage. In view of this It is imperative to explore the antioxidant potential of eye drop, as the suppression of Oxidative stress, plays an important role in dry eye syndrome besides the management of symptoms due to deficient tear film components.

The study aims at In vitro biochemical antioxidant screening comprising of Nitric oxide radical scavenging assay, ABTS radical scavenging assay, DPPH radical scavenging assay and Ferric reducing antioxidant potential (FRAP) assay

3. Materials and Methods: Determination of antioxidant potential was done adopting the following In *vitro* biochemical assays.

1. **Inhibition of Nitric oxide radical:** Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions. This was measured by the Griess reaction (Green et al., 1982; Marcocciet al., 1994).

The reaction mixture (300µl) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and eye drops and the reference compound in different concentrations (10, 25, 50, 75 and 100 µg) were incubated at 25°C for 150 min. Each 30 min, 50µl of the incubated sample was removed and 50µl of the Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediaminedi hydrochloride in 2% H_3PO_4) were added.

The absorbance of the chromophore formed was measured at 546 nm on ELISA plate reader (Bio-Tek). All the tests were performed in triplicate and the results averaged. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples. Ascorbic acid served as a positive control compound (Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JK, Tannenbaum SR. (1982). Analysis of nitrate, nitrite and 15N in biological fluids. Anal Biochem, 126: 131-136.;Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. (1994). The nitric oxide scavenging property of Ginkgo biloba extract EGb 761. Biochem Biophys Res Commun, 201:748-55.)

2. ABTS radical cationdecolourisation assay: In this assay, the oxidant is generated by persulfate oxidation of 2, 2,-azinobis (3-ethylbenzoline-6-sulfonic acid)-($ABTS^{2-}$) as described by Re et al., (1999). ABTS radical cation ($ABTS^{+}$) are produced by reacting ABTS solution (7mM) with 2.45 mm ammonium per sulphate and the mixtures were allowed to stand in dark at room temperature for 12-16 hr before use. After 16hr, this solution was diluted with ethanol until the absorbance reaches 0.7 ± 0.02 at 734 nm. For the study, 100µl of eye drops (120µg/ml) were added to 200µl of ABTS solution. The absorbance was read at 745nm and the percentage inhibition calculated. (Re R, Pellegrini N, Protoggenete A, Pannala A, Yang M, Rice-Evans C. (1999). Antioxidnat activity applying an improved ABTS radical cation decoloration assay. Free Radic Biol Med, 26: 1231-1237.)

3. Inhibition of DPPH radical: The free radical scavenging activity of eye drops was measured by 1; 1-diphenyl-2-picryl-hydrazil (DPPH) uses the method of Blois (1958) and Gomez-Alonso et al., (2003). 0.1 mM solution of DPPH in methanol was prepared and 100 µl of this solution was added to 100 µl of eye drops and the reference compound (50, 100, 150, 200 and 250 µg). After 30 min, absorbance was measured at 517 nm. Butylated Hydroxy Anisole (BHA) was used as the reference material. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. (Gomez-Alonso S, Fregapane G, Salvador MD, Gordon MH. (2003). Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. J Agric Food Chem, 51: 667-672.)

4. Reducing power/Ferric reducing antioxidant potential (FRAP) assay: The reducing power of eye drops was determined according to the method of Oyaizu (1986). 100 µl of eye drops were mixed with phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixture was incubated at 50ºC for 20 min.

A portion of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000g for 10 min. The upper layer of the solution (100 µl) was mixed with distilled water (50µl) and Ferric chloride ($FeCl_3$) (100 µl, 0.1%) and the absorbance was measured at 700 nm. Butylated Hydroxy Toluene (BHT) was used as the reference material.

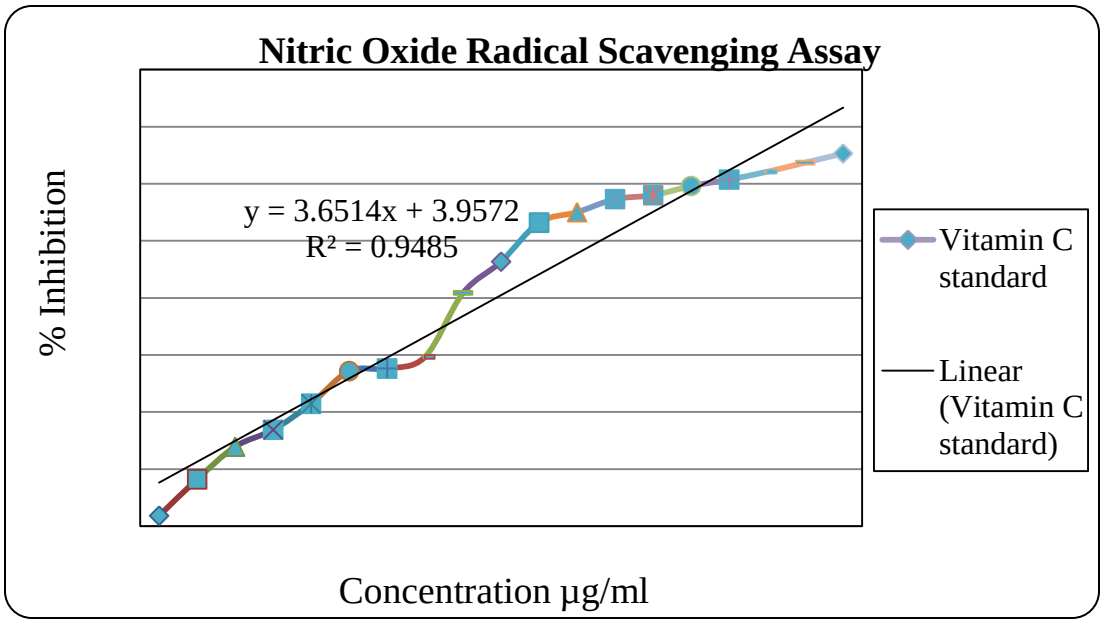
All the tests were performed in triplicate and the graph was plotted with the average of three observations.(Blois MS. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181: 1199-1200.;Oyaizu M. (1986). Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition, 44: 307-315.)

Table 1: Inhibitory Concentration (IC_{50}) obtained from the standard graphs of various assays are expressed in terms of their standard compounds.

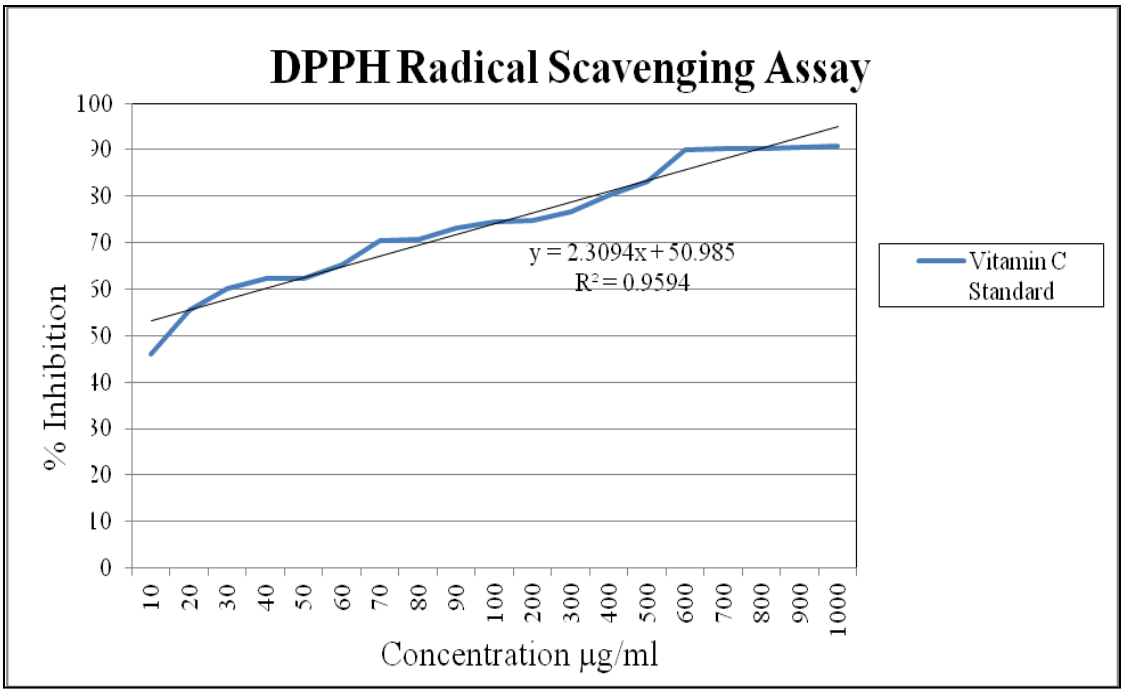
Sl. No.	Results	Sample volume of eye drops solution 120µg/ml	IC_{50} values equivalent to
1.	Nitric Oxide Radical Scavenging Assay	100µl	632.99 µg/ml of Vitamin C Standard
2.	ABTS Radical Scavenging Assay	100µl	527.70 µg/ml of Gallic Acid Standard
3.	DPPH Radical Scavenging Assay	100µl	719.32 µg/ml of Vitamin C Standard
4.	Ferric reducing antioxidant potential (FRAP) Assay	100µl	276.69 µg BHT/ml

4. Results and discussion : In vitro biochemical antioxidant assays such as Nitric oxide radical scavenging assay, **ABTS** radical scavenging assay, DPPH radical scavenging assay and Ferric reducing antioxidant potential (FRAP) assay have confirmed the antioxidant potential of the eye drop. The Inhibitory Concentration (IC_{50}) obtained from the standard graphs of various assays are expressed in terms of their standard compounds **(Table-1)(Graph-1, Graph 2, Graph.3 and Graph 4,)**

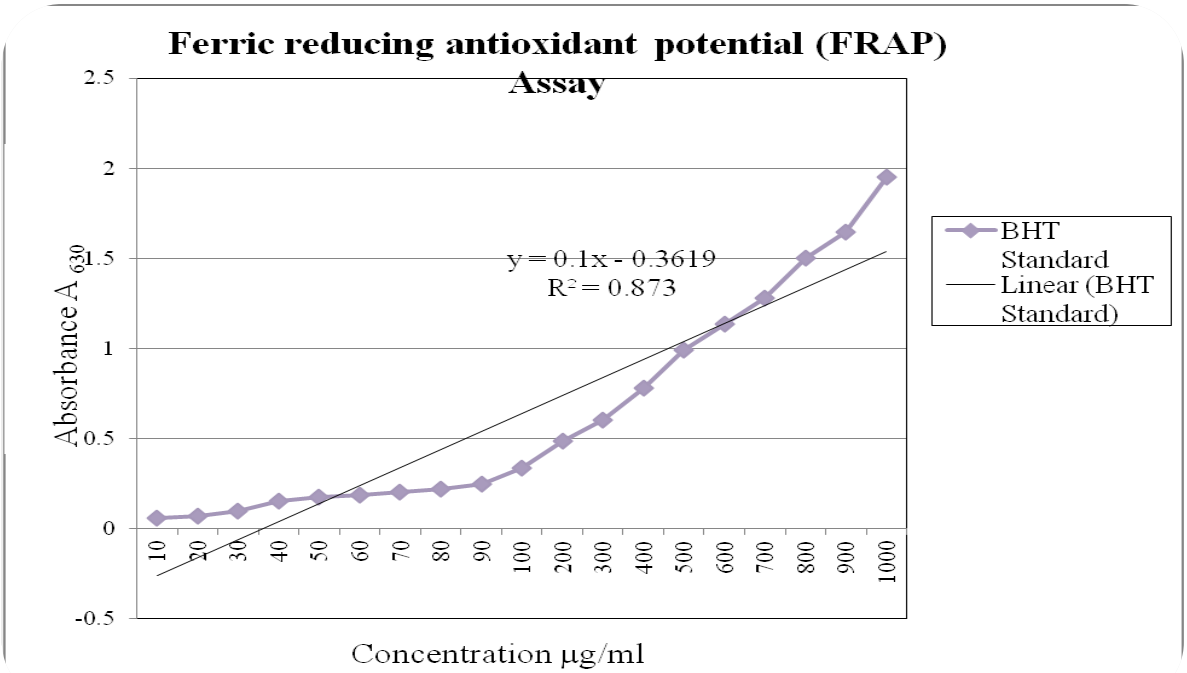
Graph 1: Nitric oxide radical scavenging assay obtained with vitamin C standard



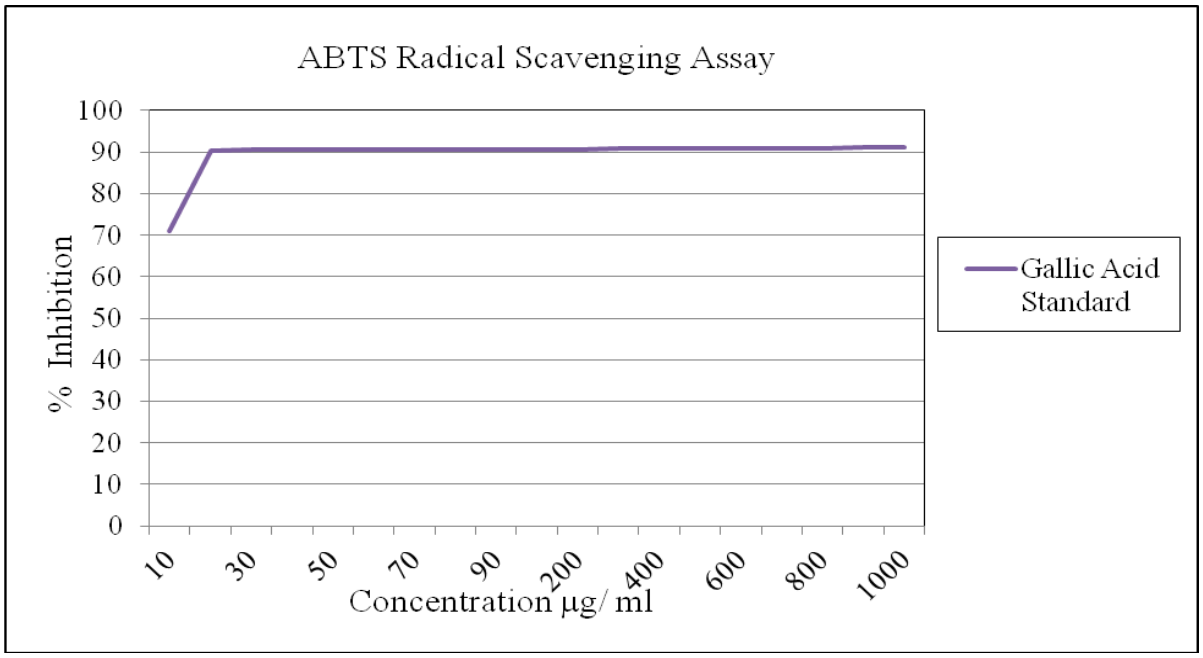
Graph 2. DPPH radical scavenging standard assay obtained with vitamin C standard.



Graph 3: FRAP standard assay obtained with BHT standard



Graph. 4: ABTS radical scavenging assay with gallic acid standard



4. Conclusion: In addition to the management of symptoms related to deficiency of tear components, prevention of damage due oxidative stress, arrest of further progress and control of infection also forms an vital component in dry eye disease. The close relationship between ocular surface epithelia and the preocular tear film ensures ocular surface health. As such, dysfunctional protective elements that lead to ocular surface and tear disorders are heterogeneous, effective therapeutic strategies are the need of hour to tackle tear disorders attributed with diverse factors. The ocular toxicity studies of standardized herbal eye drops revealed its safety on topical ophthalmic use. Further the antioxidant and anti-microbial property may contribute to effective symptom management and extenuation of basic pathology linked with tears component deficiency. The eye drops developed rationally taking potential leads from codified Ayurvedic texts probably contribute by offering comprehensive management for dry eye syndrome.

Chapter- 5
Clinical Studies

Chapter-5: Clinical Studies

The Clinical study is an interventional, randomized control, open label prospective trial with efficacy as the end point .Additionally the safety aspects viz. ADRs -Adverse Drug Reaction) and AEs(Adverse Events) have documented. the study was designed and outcome of end points such as clinical symptoms, subjective parameters and other diagnostic tests have been be subjected to Univariate and multivariate analysis using Statistical Package for Social Sciences (SPSS) 15.0versionwith appropriate statistical methods. The scoring of criteria of assessment was analyzed statistically in terms of mean value of BT (before treatment), AT (after treatment), SD (standard deviation), SE (standard error). Paired t test was applied for test of significance at $P<0.05$ and $P<0.001$**.** The study was conducted at Ayurveda Central Research Institute, New Delhi, after obtaining clearance of IEC (Institutional Ethics Committee) and registration of CTRI(Clinical Trial Registry of India).

5.1. Materials and Methods

1. Objectives

1. To evaluate the clinical efficacy of‘ DY Eye drops’ {prepared with *Daruharidra* (*Berberis aristata* DC.) & *Yastimadhu* (*Glyeyrrhiza glabra* Linn.) in Dry Eye Syndrom*e (Shushkakshi pak*a)
2. To compare the efficacy of ‘DY Eye drops’ with Artificial tears (conventional control intervention-Tear supplement- Carboxy methyl cellulose)

2. Methods

Study Type	:	Interventional
Purpose	:	Treatment
Masking	:	Open label
Control	:	Randomized controlled
Timing	:	Prospective
End Point	:	Efficacy
No. of Groups	:	Two
Sample size	:	150(75 participants each in test and control group)

3. Drug Interventions

Group-I: Installation of 'DY Eye drops' {{prepared with *Daruharidra* (*Berberis aristata* DC.) & *Yastimadhu* (*Glyeyrrhiza glabra* Linn.) three drops for three times a day for one month

Group-II: Installation of 'Artificial tears (conventional Control -Tear supplement-Carboxy methyl cellulose) three drops for three times a day for one month

4. Study Participants

Inclusion Criteria

1. Subjects of both the gender aged between 35 to 70 years.
2. Patients presenting with any of the signs and symptoms of Dry eye syndromeviz Feeling of dryness in the eyes, burningsensation, foreign body sensation (Sandy/Scratchy / itching),pricking pain, Rough lids / mucoid discharge/mild blepharitis ,stuck eyelids, blurred vision, redness) with
    - Schirmer-I test positive i.e. < 10 mm.
    - Tear film break-up time less than 10 seconds.
    - Rose Bengal staining showing devitalized epithelium of conjunctiva and mucus plaques on the cornea.

    (If any two of the above three criteria are present; the diagnosis of Dry Eye Syndrome is confirmed)
3. Willing and able to participate in the study for 4 weeks.

Exclusion Criteria

1. Severe cases of dry eye syndrome with complications like perforated corneal ulcer, Uveitis, Glaucoma etc.
2. Associated with Inflammatory conditions like acute conjunctivitis etc.
3. Systemic diseases causing Dry Eye Syndrome.
4. Pregnant / lactating females
5. Patients on steroids, oral contraceptive pills, estrogen replacement therapy or any other medication that may adversely affect the outcome of the study
6. Patients suffering from major systemic illness necessitating long term drug treatment(Rheumatoid arthritis, Psycho-Neuro- Endocrinal disorders, etc.).
7. Any other condition which the Investigator thinks may jeopardize the study.

5. Assessment

Outcomes

1. Primary Outcome Measure

- Change in Clinical Parameters-

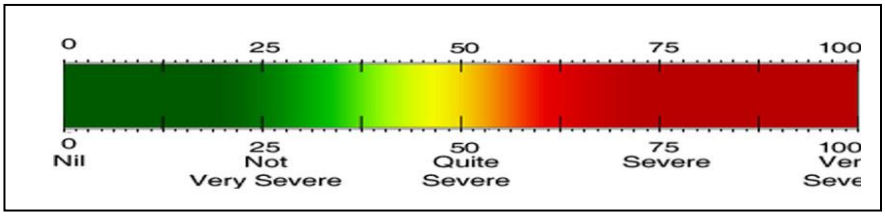
2. Secondary Outcome Measures

- Change in the Tear Film break up time
- Change in Schirmer-I Test
- Change in Rose Bengal Staining.

Assessment Parameters

(1) **Clinical Parameters**

Visual Analogue Scale



Parameters Visual analogue Scale Score

a) Feeling of Dryness in the eyes
b) Burning sensation
c) Foreign body sensation (Sandy/Scratchy / itching)
d) Pricking pain
e) Rough lids / Mucoid discharge/Mild Blepharitis
f) Stuck eyelids
g) Blurred vision
h) Inflammation / Redness
i) Narrowing of palpebral aperture

(2)**Eye Tests:**

- Tear Film break up time _____sec.
- Schirmer's I Test _____mm
- Rose Bengal Staining

Criteria for assessment of the outcome : Grading and scoring pattern was adopted for assessing following criteria before and after intervention phase.

a. Subjective presence of symptoms (Progression or regression) as per VAS
b. Objective presence of signs (Progression or regression). Following system of grading was used for recording their readings:

1. Schirmer I test

0 - Schirmer strip wetting of >15mm in 5 minutes
1 - Schirmer strip wetting between 11-15 mm in 5 minutes
2 - Schirmer strip wetting between 5-10mm in 5 minutes
3 - Schirmer strip wetting of < 5 mm in 5 minutes

2. Tear film Break Up Time

0 - The appearance of dry spot after 15 seconds
1 - The appearance of dry spot between 11-15 sceonds
2 - The appearance of dry spot between 5-10 seconds
3 - The appearance of dry spot within 5 seconds

3. Rose Bengal staining (Oxford scheme of scoring)

0 - No staining.
1 - Mild staining. (Dot count 10)
2 - Moderate staining (Dot count 32)
3 - Moderately Severe staining (Dot count 100)
4 - Intense staining (Dot count 316)

c. Clinical Tests

i. Schirmer I test: Increase or decrease in wetting of strips in 5 minutes.
ii. TBUT: Increase or decrease in tear film break up time
iii. Rose Bengal staining: Absence, decrease or increase in conjunctival and corneal staining.

5. Withdrawal Criteria

- The participant may be withdrawn from the trial if –during the course of the trial treatment, if any serious condition develops/ symptoms aggravate, which requires urgent treatment, necessitating the institution of new modalities of treatment.

or

- Non-compliance of the treatment regimen (minimum 80% compliance is essential to continue in the study).

7.Statistical Methods: Clinical symptoms, Subjective parameters and clinical test outcomes be have been subjected to Univariate and multivariate analysis using Statistical Package for Social Sciences (SPSS) 15.0versionwith appropriate statistical methods.The scoring of criteria of assessment was analyzed statistically in terms of mean value of BT (before treatment), AT (after treatment), SD (standard deviation), SE (standard error). Paired t test was applied for test of significance at $P<0.05$ and $P<0.001$**.**

Adverse Events: Any untoward medical occurrence that may present during the treatment with a pharmaceutical product but which does not necessarily have a causal relationship with this treatment.

Adverse Reactions (ADRs):A response which is noxious and unintended, and which occurs at doses normally used in humans for the prophylaxis, diagnosis, or therapy of disease, or for the modification of physiological function.(WHO, 1972).

Drug Compliance : If there is more than or equal to 80% compliance, the participant would be continued in thetrial. The compliance needs to be assessed at each visit during the follow up (i.e. Day 14th, 28th, by counting the number of empty containers consumed by the participant.

Concomitant Medication: A concomitant medication (con-med) is a drug or biological product, other than a study drug taken by a subject during a clinical trial. Participants registered under the trial will be issued treatment cards with the entire treatment regimen written on it. They will be instructed to avoid the use of any otherdrugs on their own for any ailment and will be clearly instructed to consult the treating Investigator for any symptom or complaint, or if they feel anything unusual. The Investigator will record any medication(s) he / she may prescribe to alleviate their ailments**.**

Rescue Medication: Rescue medication / Quick-acting medication / Fast-acting Medication - A medication intended to relieve symptoms immediately. This is in contrast to preventive medications, which are taken over a long period of time to prevent or manage symptoms. To alleviate any emergency, the use of rescue medication is permitted

as per the wisdom / discretion of the Investigator. However, the same need to be documented in appropriate column in the Case report form.

Institutional Ethics Committee (IEC):. Written approval of IEC has been obtained from IEC according to the usual procedure.

Chronological schedules of Assessments (Table-1)

Prior to selection – (Screening)

a) Informed Consent
b) Eligibility evaluation
c) Tear film break-up time
d) Schirmer - I test
e) Rose Bengal staining

During Selection - (Baseline)

a) General information-(Personal Identification and Demographic profile)
b) Medical history , General Physical and Systemic examination
c) Tear film break-up time (carry forward from screening)
d) Schirmer-I test (carry forward from screening)
e) Rose Bengal staining (carry forward from screening)
f) Clinical Assessment.
g) Assessment of Ayurvedic Parameters.
h) Issue of drugs and drug compliance report form
i) Instructions to come after 2 weeks (14 days).

During Treatment i.e. on 14th day

a) Physical examination.
b) Clinical assessment
c) Tear film break-up time
d) Schirmer-I test
e) Rose Bengal staining
f) Assessing drug compliance
g) Issue of drugs and drug compliance report form
h) Instructions to come after 2 weeks (14 days).

At the end of the treatment i.e. at the end of 4th week (28th day):

a) Clinical Assessment.

b) Assessment of Ayurvedic Parameters.

c) Tear film break-up time.

d) Schirmer-I test

e) Rose Bengal staining.

f) Laboratory Investigations.

g) Assessing drug compliance.

Chronological schedules of Assessments

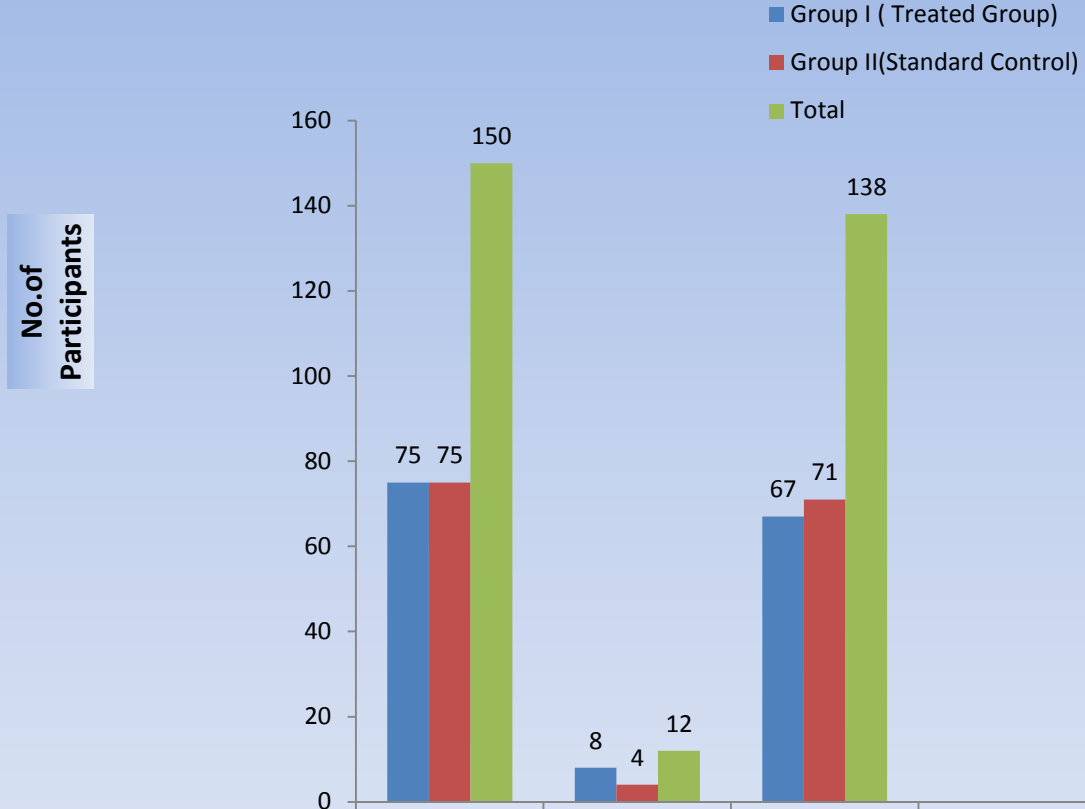
Components of assessment	Screening	Baseline	At the end of 14th day	At the end of4weeks (28th day).
Informed consent	■			
Demographics and medical history		■		
Investigations	■			■
Clinical Examination		■	■	■
Assessment of Ayurvedic parameters		■		■
Concomitant medication			■	■
Rescue medication			■	■
• **Tear film break-uptime.** • **Schirmer-I test** • **Rose Bengal staining**	■		■	■
Assessment of ADRs / AEs			■	■
Assessment of Drug compliance			■	■
Issue of drugs and Drug Compliance Report Form every 2 week (14 days).		■	■	

5.2. Observations and Results

1. Observations

Enrollment and adherence of study participants : 150 enrolled participants were randomly distributed into 2 groups, **Group-1(DY-Drops) and Group-II standard control (Carboxy methyl cellulose)** each comprising of 75 subjects .Out of 150 recruited eligible participants, 138 (92%) subjects completed the study and there were 12 (8%) dropouts. In Group-I, 67 participants completed the study and 8 were dropouts while in Group-II, 71 completed the study with 4 dropouts**.(Table-1,Graph-1)**

GRAPH-1.Details of Enrolled(n=150),Dropouts (n=12)and Completed(n=138) Study Participants in Treated and Control Groups



	Enrolled cases	Drop outs	Completed Cases	
Group I (Treated Group)	75	8	67	
Group II(Standard Control)	75	4	71	
Total	150	12	138	

Table-1.Details of Enrolled and Completed Study Participants in Treated and Control Groups

Trial groups	Enrolled Cases	Dropouts	Completed Cases
Group I (Treated Group)	75	8	67
Group II(Standard Control)	75	4	71
Total	**150**	**12(8%)**	**138(92%)**

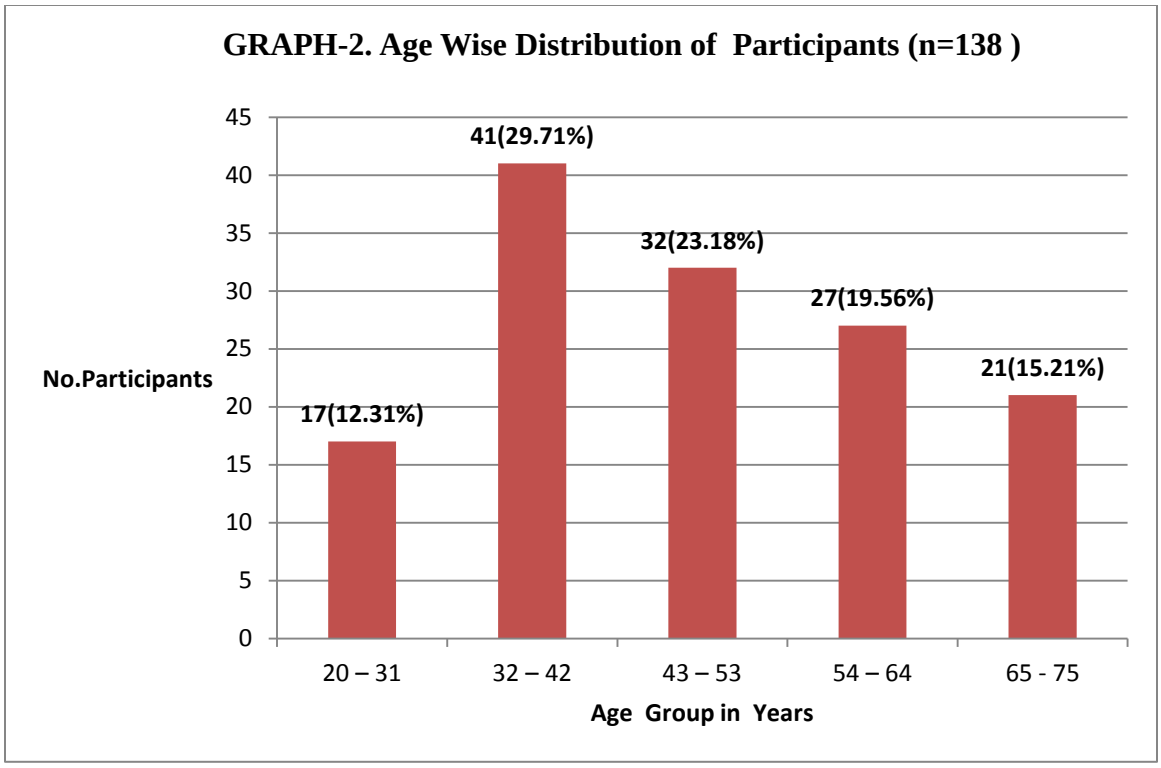
`

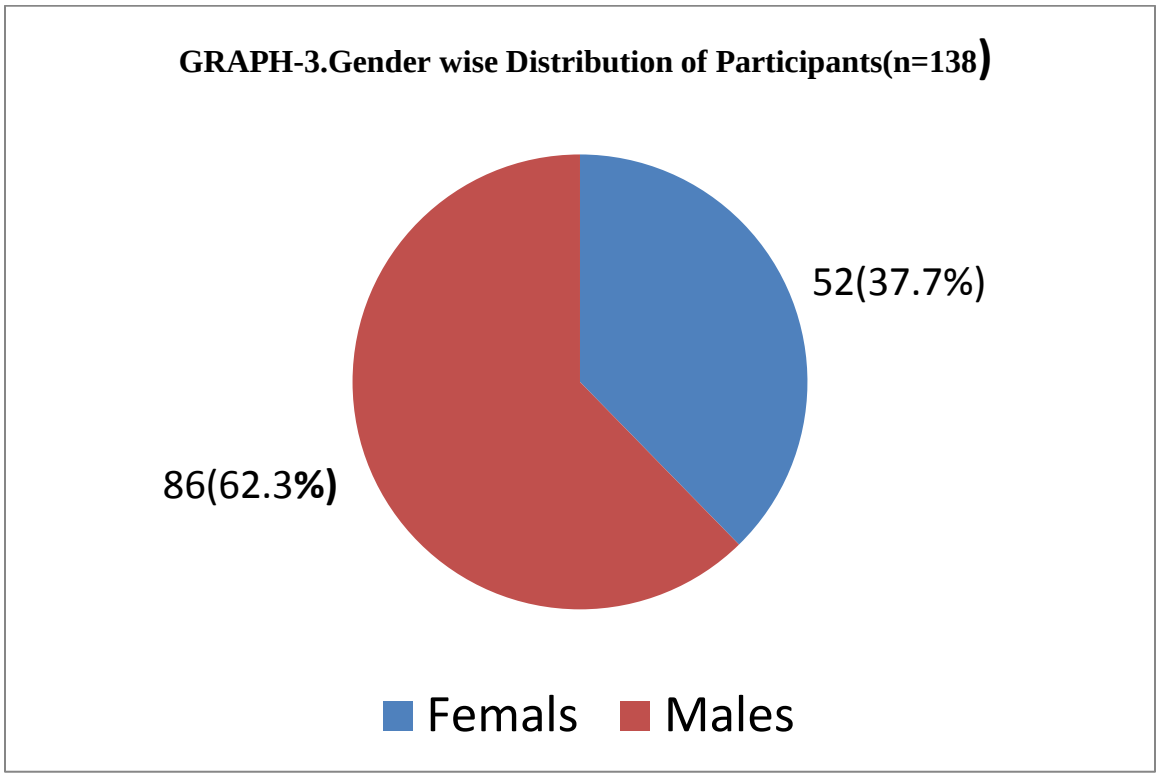
Demographic Profile: The demographic data has been presented for 138 participants whereas clinical examination findings have been detailed for individual eye amounting to 276 eyes. The demographic data was collected and grouped on the basis of various parameters such as age, gender, prakriti, religion, educational status, dietary habits , addictions etc.

Age and Gender: Maximum numbers of participants were in present study belonged to age groups of 32-42 years (29.71%)and 43-53 (23.18%) accounting for 52.89% of the total 138 registered participants followed by participants belonging to age group 54-64years (19.56%). In present study, 37.7% participants were females and 62.3% % participants were males.(**(Table-2, Graph-2 and Graph-3)**

Table-2Age and Gender wise distribution of completed participants

Age group	Gender wise Distribution of Participants(Number)		Total	Percentage
	Female	Male		
20 - 31	5	13	17	12.31%
32 – 42	13	28	41	29.71%
54 – 64	15	12	27	19.56%
65 – 75	10	11	21	15.21%
Total	**52**	**86**	**138**	**100%**
	37. 7%	**62.3%**	**100 %**	





Religion and Education Status : Majority of participants registered belonged to Hindu religion i.e. 86.2%(119) while 8.69% (12)were followers of Islam followed by 5.07% (5)participants who were Christians .84% (116)participants were educated up to graduation followed by 15.94% (22) who completed up to higher secondary education. Most of the completed participants in the study were married i.e. 98.55%(136) . .

Work profile and Occupation: The work profile and occupation comprise of 56.52 % (78)of the participants belongs to service group 31.8% (43) were house makers followed by 12.31% (17) were engaged in business. The socio-economic status of participants revolve that 10.9 % (15) of participants possess BPL cards while other 89.1% (123) falls either in middle and higher economic status .

Disease course: Concerning the onset and course of symptoms, 90.75%(125) participants had chronic and recurrent nature of the dry eye while 9.42% (13) presented with acute onset

Dietary profile: Further the dietary profile of participants reflect vegetarian predominance with 59.42%(82) while 40.57%(56) had mixed diet habits.

Prakriti: Most of the study participants were of *vata*-predominant *prakriti* with 71.01%(98) followed by 15.94% (22) *pitta* predominant *prakriti* and 13.04% (18) participants were of *kapha* predominant *prakriti*.

Visual acuity: Observations of Visual acuity of participants comprise 6/6 in 9.42%(13), , 6/9 in 23.91%(33)6/12 in 18.84%(26),6/18 in 11.59%(16),6/24 in 19.56%(27),6/36 in 10.14%(14) and 6/60 or less was observed in 6.51%(9) participants.

5.2. Results

Effect of the therapy was assessed in completed study participants in study groups treated with DY eye drops (Group-1) and standard control (conventional Control tear supplement-Carboxy methyl cellulose) (Group-II) by analyzing the outcomes subjective and objective parameter before and after the scheduled interventions**.**

Intervention Schedules : Group-I participants were treated with Installation of 'DY Eye drops' prepared with *Daruharidra*(*Berberis aristata*) *Yastimadhu*(*Glyeyrrhiza glabra*) 3 drops for 3 times a day for one month while Installation of conventional Control tear supplement- (Carboxy methyl cellulose) 3 drops for 3 times a day for same period for participants in Group-II

Assessment schedules: The Clinical assessment subjective parameters(progression or regression) viz. the changes inBlurred Vision, Feeling of dryness, Burning sensation, Foreign Body Sensation, Narrowing of aperture, Pricking pain, Redness, Rough Lids, Stuck eyelids were assessed using Visual Analogue Scale (VAS) along with objective assessment of Tear film break-up time(TUBT),Schirmer-I test, Rose Bengal staining was done at baseline(0 -day), 14^{th} day and 28^{th} day. Further ,the assessing of drug compliance, inquiry on ADRs(Adverse Drug Reaction) and AEs(Adverse Events) were performed as per schedule.

Statistical Methods: Clinical symptoms, Subjective parameters and objective are subjected to Univariate and multivariate analysis using Statistical Package for Social Sciences (SPSS) 15.0versionwith appropriate statistical methods. The scoring of criteria of assessment was analysed statistically in terms of mean value of BT (before treatment), AT (after treatment), SD (standard deviation), SE (standard error). Paired t test was applied for test of significance at $P<0.05$ and $P<0.001$**. (Table-3 to 37 (Graphs-4 to 19)**

A. Subjective Parameters

1. Blurred vision: In DY drops treated group **(Group-I)** the mean value of in VAS observed at baseline (0.day),before treatment (BT)) was 61.02 and it was 17.71 while after treatment at 28th day (AT) **(p-value=<0.001).** In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value of in VAS observed at baseline (0.day),before treatment (BT)) was 61.54 and it was 20.19 while after treatment at 28th day (AT) **(p-value=<0.001).**

The mean value in reduction of symptoms in test intervention (DY Drops) is comparable to conventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant. **(p-value=<0.001).** **(Tables-3, 4,5 and 33) (Graphs- 12 ,13)**

Table-3. Mean values of Changes in 'Blurred vision Before and After treatment in Treatment Group-I (n=67)

Blurred Vision	Mean	N	Std. Deviation	Std. Error Mean
1st day	63.02	48	19.966	2.882
14th day	31.77	48	20.460	2.953
28th day	17.71	48	16.274	2.349

Blurred Vision	Paired Differences					t	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	31.250	24.462	3.531	24.147	38.353	8.851	47	<0.001
1st day - 28th day	45.313	22.864	3.300	38.673	51.952	13.730	47	<0.001

Table-4. Mean values of Changes in 'Blurred vision Before and After treatment in Treatment Group-II (n=71)

Blurred Vision	Mean	N	Std. Deviation	Std. Error Mean
1st day	61.54	52	18.164	2.519
14th day	35.58	52	17.395	2.412
28th day	20.19	52	14.884	2.064

Blurred Vision	Paired Differences					t	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	25.962	20.982	2.910	20.120	31.803	8.923	51	<0.001
1st day - 28th day	41.346	20.343	2.821	35.683	47.010	14.656	51	<0.001

Table-5. Mean values of Changes in 'Blurred vision Before and After treatment in Group-I and Group-II (n=138)

Independent sample t test

Blurred Vision	Group	N	Mean	Std. Deviation	Std. Error Mean
Diff of Baseline and 28th day	**1**	48	45.31	22.864	3.300
	2	52	41.35	20.343	2.821

	t-test for Equality of Means						
	t-value	df	p-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower Bound	Upper Bound
Diff of Baseline and 28th day	.918	98	.361	3.966	4.321	-4.609	12.542

2. Feeling of dryness: In DY drops treated group **(Group-I)** the mean value of in VAS observed at baseline (0.day),before treatment (BT)) was 71.88 and it was 17.71 while after treatment at 28th day (AT) **(p-value=<0.001).**

In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value of in VAS observed at baseline (0.day),before treatment (BT))was 68.27 and it was 15.38 while after treatment at 28th day (AT) **(p-value=<0.001).**

The mean value in reduction of symptoms in test intervention (DY Drops) is comparable toconventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant. **(p-value=<0.001). (Tables-6,7,8, and 33) (Graphs-4 ,12 and 13)**

GRAPH-4.Mean values of Changes in 'Feeling of dryness' Before and After treatment (n=138)

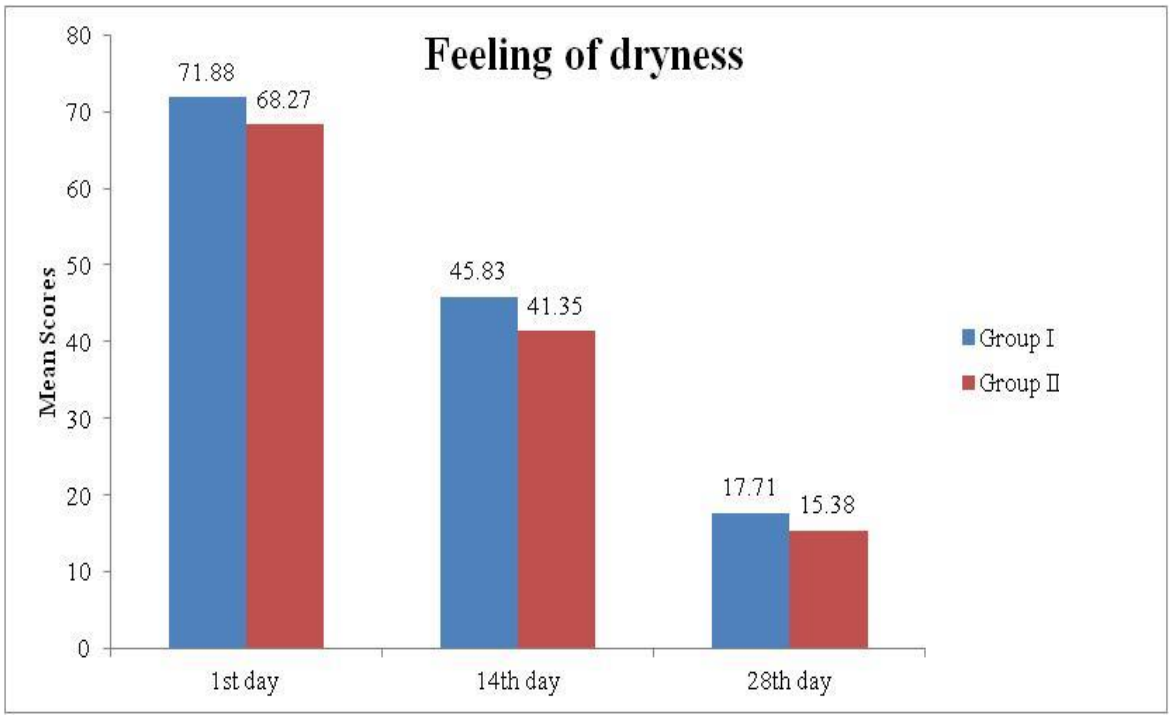


Table-6. Mean values of Changes in 'Feeling of Dryness' Before and After treatment in Treatment Group-I (n=67)

Feeling of dryness	Mean	N	Std. Deviation	Std. Error Mean
1st day	71.88	48	14.241	2.055
14th day	45.83	48	15.755	2.274
28th day	17.71	48	14.548	2.100

Feeling of dryness	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	26.042	13.604	1.964	22.092	29.992	13.263	47	<0.001
1st day - 28th day	54.167	15.755	2.274	49.592	58.742	23.819	47	<0.001

Table-7. Mean values of Changes in 'Feeling of Dryness' Before and After treatment in Treatment Group-II (n=71)

Feeling of Dryness	Mean	N	Std. Deviation	Std. Error Mean
1st day	68.27	52	17.929	2.486
14th day	41.35	52	17.771	2.464
28th day	15.38	52	13.242	1.836

Feeling of Dryness	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	26.923	10.899	1.511	23.889	29.957	17.814	51	<0.001
1st day - 28th day	52.885	18.952	2.628	47.608	58.161	20.123	51	<0.001

Table-8. Mean values of Changes in 'Feeling of Dryness' Before and After treatment in Group-I and Group-II (n=138)
Independent sample t-test

Feeling of dryness	Group	N	Mean	Std. Deviation	Std. Error Mean
Diff of Baseline and 28th day	**1**	48	54.17	15.755	2.274
	2	52	52.88	18.952	2.628

Feeling of dryness	t-test for Equality of Means						
	t-value	df	p-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower Bound	Upper Bound
Diff of Baseline and 28th day	.366	98	.715	1.282	3.501	-5.666	8.230

3. Burning sensation: In DY drops treated group **(Group-I)** the mean value of in VAS observed at baseline (0.day),before treatment (BT)) was 54.69 and it was 6.25 while after treatment at 28th day (AT) **(p-value=<0.001).**

In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value of in VAS observed at baseline (0.day),before treatment (BT))was 57.21 and it was 5.29 while after treatment at 28th day (AT) **(p-value=<0.001).**

The mean value in reduction of symptoms in test intervention (DY Drops) is comparable toconventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant. **(p-value=<0.001).(Tables-9,10,,11,and 33) (Graphs.5 ,12 and 13)**

GRAPH-5.Mean values of Changes in 'Burning sensation' before and After treatment (n=138)

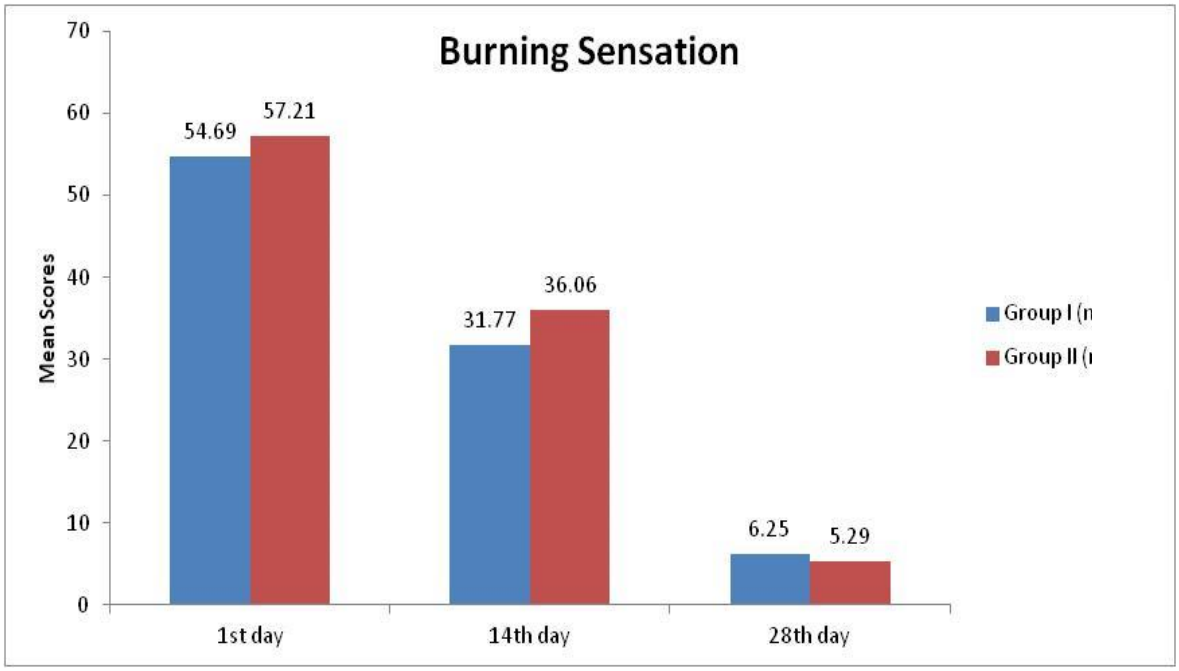


Table-9. Mean values of Changes in 'Burning Sensation' Before and After treatment in Treatment Group-I (n=67)

Burning sensation	Mean	N	Std. Deviation	Std. Error Mean
1st day	54.69	48	26.122	3.770
14th day	31.77	48	21.721	3.135
28th day	6.25	48	10.940	1.579

Burning sensation	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	22.917	16.172	2.334	18.221	27.612	9.818	47	<0.001
1st day - 28th day	48.438	23.860	3.444	41.509	55.366	14.065	47	<0.001

Table-10. Mean values of Changes in 'Burning Sensation' Before and After treatment in Treatment Group-II (n=71)

Burning sensation	Mean	N	Std. Deviation	Std. Error Mean
1st day	57.21	52	24.920	3.456
14th day	36.06	52	20.057	2.781
28th day	5.29	52	11.437	1.586

Burning sensation	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	21.154	15.166	2.103	16.932	25.376	10.058	51	<0.001
1st day - 28th day	51.923	24.677	3.422	45.053	58.793	15.173	51	<0.001

Table-11. Mean values of Changes in 'Burning Sensation' Before and After treatment in Group-I and Group-II (n =138)

Independent Sample t-test

Burning sensation	Group	N	Mean	Std. Deviation	Std. Error Mean
Diff of Baseline and 28th day	**1**	48	48.44	23.860	3.444
	2	52	51.92	24.677	3.422

Burning sensation	t-test for Equality of Means						
	t-value	df	p-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower Bound	Upper Bound
Diff of Baseline and 28th day	.717	98	.475	-3.486	4.862	-13.133	6.162

4. Foreign Body Sensation: In DY drops treated group **(Group-I)** the mean value of in VAS observed at baseline (0.day),before treatment (BT)) was 51.17 and it was 4.69while after treatment at 28th day (AT) **(p-value=<0.001).**

In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value of in VAS observed at baseline (0.day),before treatment (BT))was 58.17 and it was 4.33 while after treatment at 28th day (AT) **(p-value=<0.001).**

The mean value in reduction of symptoms in test intervention (DY Drops) is comparable to conventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant**(p-value=<0.001).(Tables-12,13,,14,and 33) (Graphs-6 ,12 and 13)**

GRAPH-6.Mean values of Changes in 'Foreign sensation' Before and After treatment (n=138)

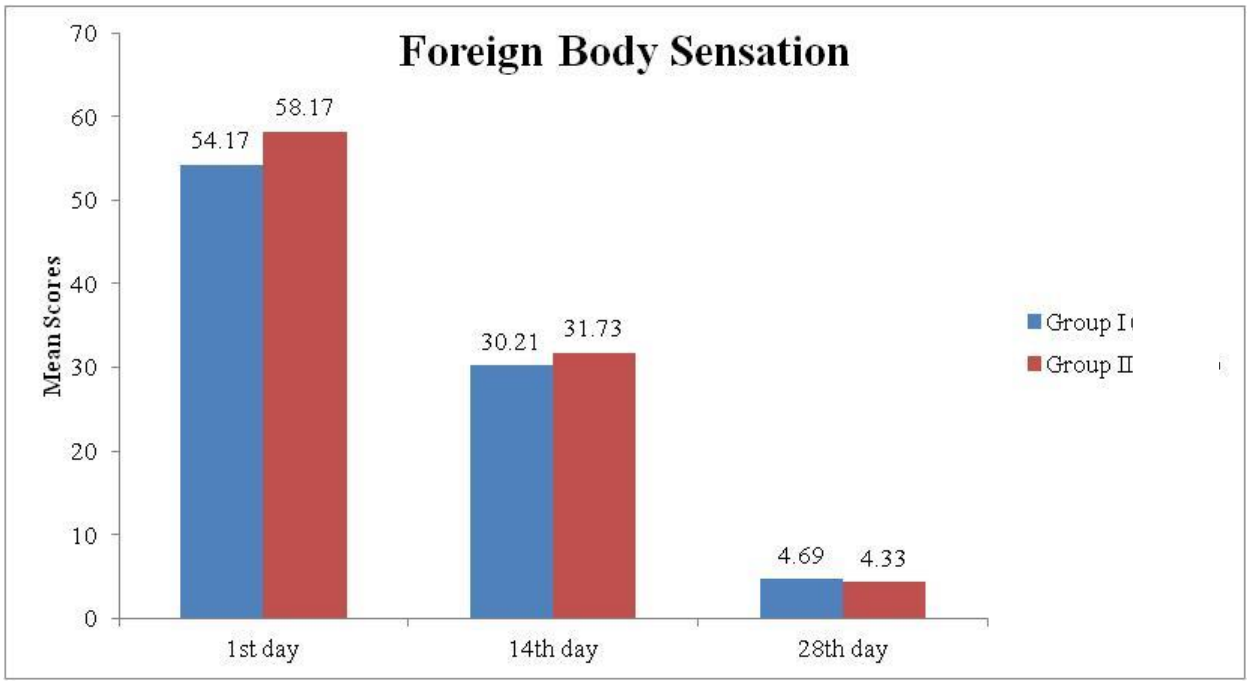


Table-12. Mean values of Changes in 'Foreign Body sensation ' Before and After treatment in Treatment Group-I (n=67)

Foreign Body Sensation	Mean	N	Std. Deviation	Std. Error Mean
1st day	54.17	48	26.962	3.892
14th day	30.21	48	21.854	3.154
28th day	4.69	48	9.861	1.423

Foreign Body Sensation	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	23.958	16.274	2.349	19.233	28.684	10.199	47	<0.001
1st day - 28th day	49.479	24.994	3.608	42.222	56.737	13.715	47	<0.001

Table-13. Mean values of Changes in 'Foreign Body sensation ' Before and After treatment in Treatment Group-II (n=71)

Foreign Body Sensation	Mean	N	Std. Deviation	Std. Error Mean
1st day	58.17	52	25.108	3.482
14th day	31.73	52	20.482	2.840
28th day	4.33	52	9.550	1.324

Foreign Body Sensation	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	26.442	18.796	2.606	21.210	31.675	10.145	51	<0.001
1st day - 28th day	53.846	23.941	3.320	47.181	60.511	16.219	51	<0.001

Table-14. Mean values of Changes in Foreign body Sensation' Before and After treatment in Group-I and Group-II (n =138)

Independent Sample -t-test

Foreign Body Sensation	Group	N	Mean	Std. Deviation	Std. Error Mean
Diff of Baseline and 28th day	1	48	49.48	24.994	3.608
	2	52	53.85	23.941	3.320

Foreign Body Sensation	t-test for Equality of Means						
	t-value	df	p-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower Bound	Upper Bound
Diff of Baseline and 28th day	.892	98	.374	-4.367	4.894	-14.079	5.346

5. Narrowing of aperture: In DY drops treated group **(Group-I)** the mean value of in VAS observed at baseline (0.day),before treatment (BT)) was 1.56 and it was 0.00while after treatment at 28th day (AT) **(p-value=<0.001).**

In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value of in VAS observed at baseline (0.day),before treatment (BT))was 6.25 and it was 0.00 while after treatment at 28th day (AT) **(p-value=<0.001).**

The mean value in reduction of symptoms in test intervention (DY Drops) is comparable toconventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant**(p-value=<0.001).(Tables-15,16,,17,and 33) (Graphs-7 ,12 and 13)**

GRAPH-7.Mean values of Changes in 'Narrowing of aperture ' Before and After treatment (n=138)

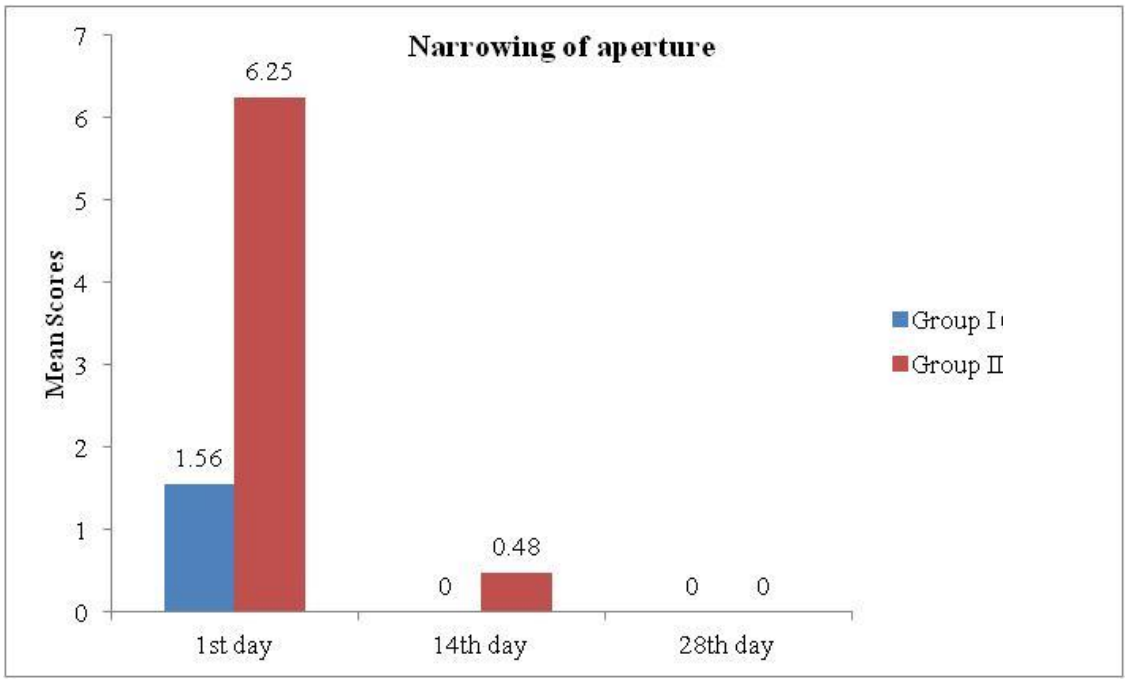


Table-15. Mean values of Changes in 'Narrowing of aperture ' Before and After treatment in Treatment Group-I (n=67)

Narrowing of aperture	Mean	N	Std. Deviation	Std. Error Mean
1st day	1.56	48	6.116	.883
14th day	.00	48	.000	.000
28th day	.00	48	.000	.000

Narrowing of aperture	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	1.563	6.116	.883	-.213	3.338	1.770	47	.083
1st day - 28th day	1.563	6.116	.883	-.213	3.338	1.770	47	.083

Table-16. Mean values of Changes in 'Narrowing of aperture ' Before and After treatment in Treatment Group-II (n=71)

Narrowing of aperture	Mean	N	Std. Deviation	Std. Error Mean
1st day	6.25	52	16.326	2.264
14th day	.48	52	3.467	.481
28th day	.00	52	.000	.000

Narrowing of aperture	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	5.769	15.352	2.129	1.495	10.043	2.710	51	.009
1st day - 28th day	6.250	16.326	2.264	1.705	10.795	2.761	51	.008

Table-17. Mean values of Changes in 'Narrowing of aperture' Before and After treatment in Group-I and Group-II (n =138)

Independent Sample t-test

Narrowing of aperture	Group	N	Mean	Std. Deviation	Std. Error Mean
Diff of Baseline and 28th day	**1**	48	1.56	6.116	.883
	2	52	6.25	16.326	2.264

Narrowing of aperture	t-test for Equality of Means						
	t-value	df	p-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower Bound	Upper Bound
Diff of Baseline and 28th day	1.871	98	.064	-4.688	2.505	-9.659	.284

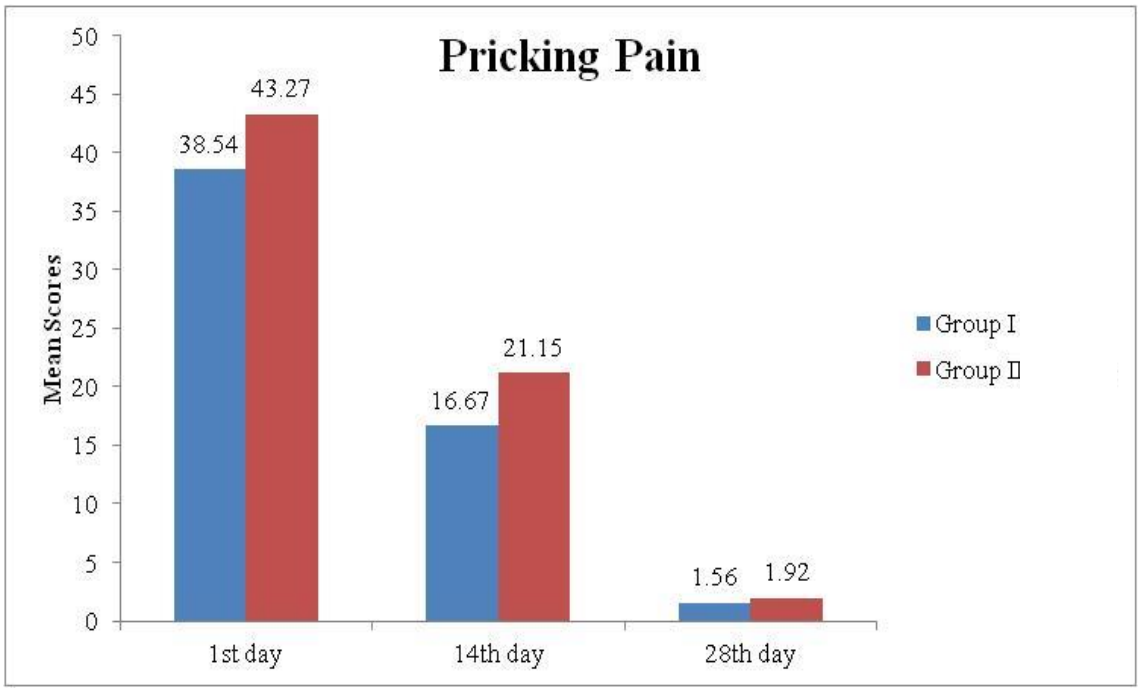
6. Pricking pain: In DY drops treated group **(Group-I)** the mean value of in VAS observed at baseline (0.day),before treatment (BT)) was 38.54 and it was 1.56while after treatment at 28th day (AT) **(p-value=<0.001).**

In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value of in VAS observed at baseline (0.day),before treatment (BT))was 43.27 and it was 1.92 while after treatment at 28th day (AT) **(p-value=<0.001).**

n=138

The mean value in reduction of symptoms in test intervention (DY Drops) is ( )le toconventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant**(p-value=<0.001).(Tables-18,19,20,and 33) (Graphs-8 ,12 and 13)**

GRAPH-8.Mean values of Changes in 'Pricking pain ' Before and After treatment (n=138)



18. Mean values of Changes in 'Pricking pain' Before and After treatment in Treatment Group-I (n=67)

Pricking pain	Mean	N	Std. Deviation	Std. Error Mean
1st day	38.54	48	27.269	3.936
14th day	16.67	48	18.831	2.718
28th day	1.56	48	6.116	.883

Pricking pain	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	21.875	21.027	3.035	15.769	27.981	7.207	47	<0.001
1st day - 28th day	36.979	25.780	3.721	29.493	44.465	9.938	47	<0.001

Table- 19. Mean values of Changes in 'Pricking pain' Before and After treatment in Treatment Group-II (n=71)

Pricking pain	Mean	N	Std. Deviation	Std. Error Mean
1st day	43.27	52	25.300	3.508
14th day	21.15	52	20.040	2.779
28th day	1.92	52	6.727	.933

Pricking pain	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	22.115	21.948	3.044	16.005	28.226	7.266	51	<0.001
1st day - 28th day	41.346	23.683	3.284	34.753	47.940	12.589	51	<0.001

Table-20. Mean values of Changes in 'Pricking pain' Before and After treatment in Group-I and Group-II (n =138)

Independent sample t-test

	Group	N	Mean	Std. Deviation	Std. Error Mean
Diff of Baseline and 28th day	**1**	48	36.98	25.780	3.721
	2	52	41.35	23.683	3.284

	t-test for Equality of Means						
	t-value	df	p-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower Bound	Upper Bound
Diff of Baseline and 28th day	.883	98	.379	-4.367	4.946	-14.183	5.449

7. Redness : In DY drops treated group **(Group-I)** the mean value of in VAS observed at baseline (0.day),before treatment (BT)) was 41.12 and it was 2.08 while after treatment at 28th day (AT) **(p-value=<0.001).**

In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value of in VAS observed at baseline (0.day),before treatment (BT))was 42.31 and it was 1.92 while after treatment at 28th day (AT) **(p-value=<0.001).**

The mean value in reduction of symptoms in test intervention (DY Drops) is comparable toconventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant**(p-value=<0.001). (Tables-21,22,23,and 33) (Graphs-9 ,12 and 13)**

GRAPH-9.Mean values of Changes in 'Redness ' Before and After treatment (n=138)

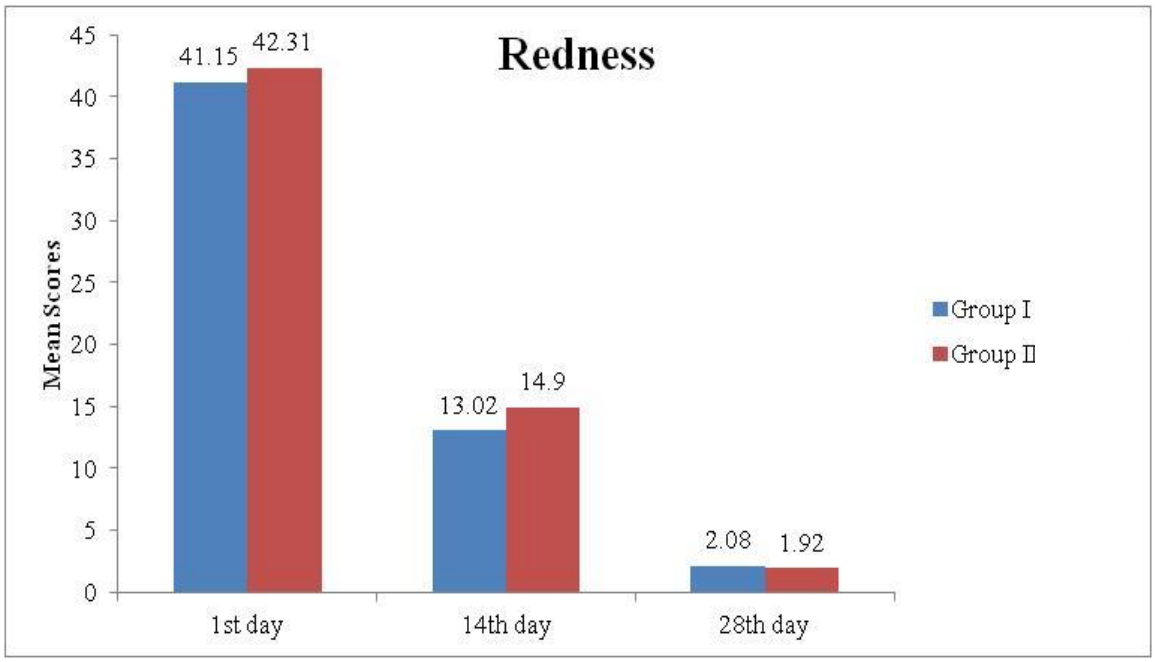


Table-21. Mean values of Changes in 'Redness' Before and After treatment in Group-I (n =67)

Redness	Mean	N	Std. Deviation	Std. Error Mean
1st day	41.15	48	26.543	3.831
14th day	13.02	48	17.096	2.468
28th day	2.08	48	6.983	1.008

Redness	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	28.125	25.066	3.618	20.846	35.404	7.774	47	<0.001
1st day - 28th day	39.063	26.751	3.861	31.295	46.830	10.117	47	<0.001

Table-22. Mean values of Changes in 'Redness' Before and After treatment in Group-II (n =71)

Redness	Mean	N	Std. Deviation	Std. Error Mean
1st day	42.31	52	23.503	3.259
14th day	14.90	52	16.612	2.304
28th day	1.92	52	6.727	.933

Redness	Paired Differences					t	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	27.404	24.882	3.450	20.477	34.331	7.942	51	<0.001
1st day - 28th day	40.385	22.770	3.158	34.045	46.724	12.789	51	<0.001

Table-23. Mean values of Changes in 'Redness' Before and After treatment in Group-I and Group-II (n =138)

Independent sample t-test

Redness	Group	N	Mean	Std. Deviation	Std. Error Mean
Diff of Baseline and 28th day	**1**	48	39.06	26.751	3.861
	2	52	40.38	22.770	3.158

Redness	t-test for Equality of Means						
	t-value	df	p-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower Bound	Upper Bound
Diff of Baseline and 28th day	.267	98	.790	-1.322	4.956	-11.157	8.512

8. Rough lids : In DY drops treated group **(Group-I)** the mean value of in VAS observed at baseline (0.day),before treatment (BT)) was 22.40 and it was 0.52while after treatment at 28th day (AT) **(p-value=<0.001).**

In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value of in VAS observed at baseline (0.day),before treatment (BT))was 26.92 and it was 0.48 while after treatment at 28th day (AT) **(p-value=<0.001).**

The mean value in reduction of symptoms in test intervention (DY Drops) is comparable to conventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant**(p-value=<0.001).(Tables-24,25,26,and 33) (Graphs-10 ,12 and 13)**

GRAPH-10.Mean values of Changes in 'Rough lids' Before and After treatment (n=138)

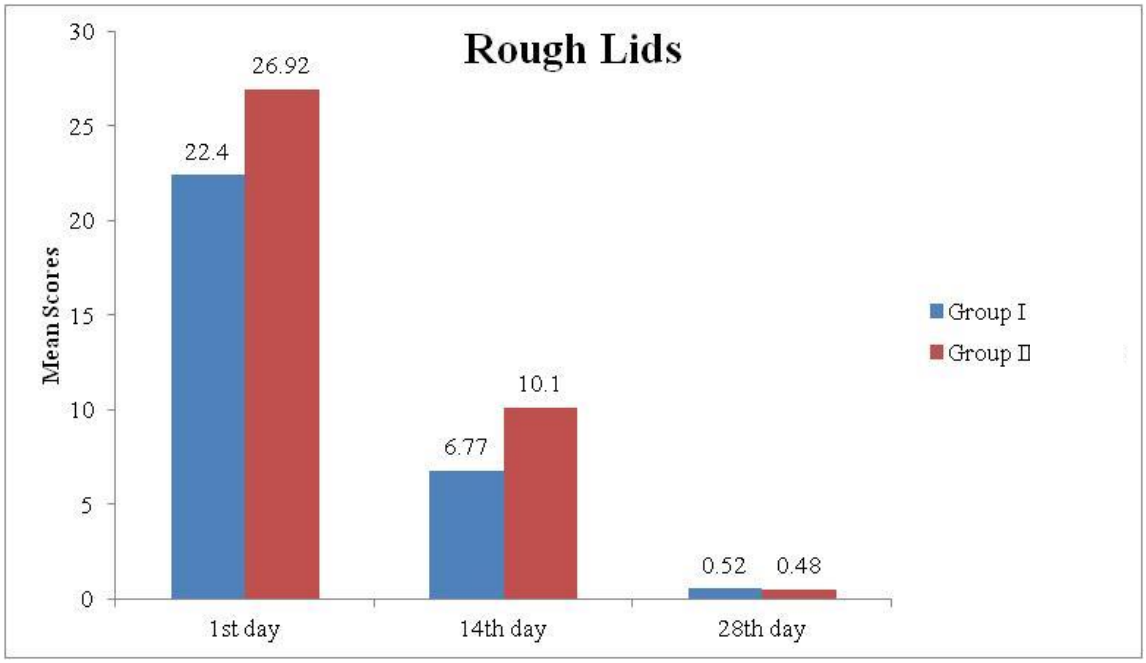


Table-24. Mean values of Changes in 'Rough lids' Before and After treatment in Group-I (n =67)

Rough Lids	Mean	N	Std. Deviation	Std. Error Mean
1st day	22.40	48	27.405	3.956
14th day	6.77	48	14.347	2.071
28th day	.52	48	3.608	.521

Rough Lids	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	15.625	21.027	3.035	9.519	21.731	5.148	47	<0.001
1st day - 28th day	21.875	26.610	3.841	14.148	29.602	5.695	47	<0.001

Table-25. Mean values of Changes in 'Rough lids' Before and After treatment in Group-II (n =71)

Rough Lids	Mean	N	Std. Deviation	Std. Error Mean
1st day	26.92	52	26.125	3.623
14th day	10.10	52	17.334	2.404
28th day	.48	52	3.467	.481

Rough Lids	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	16.827	20.244	2.807	11.191	22.463	5.994	51	<0.001
1st day - 28th day	26.442	25.921	3.595	19.226	33.659	7.356	51	<0.001

Table-26. Mean values of Changes in 'Rough lids' Before and After treatment in Group-I and Group-II (n =138)

Independent Sample t-test

	Group	N	Mean	Std. Deviation	Std. Error Mean
Diff of Baseline and 28th day	**1**	48	21.88	26.610	3.841
	2	52	26.44	25.921	3.595

	t-test for Equality of Means						
	t-value	df	p-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower Bound	Upper Bound
Diff of Baseline and 28th day	.869	98	.387	-4.567	5.255	-14.996	5.861

9. Struck lids : In DY drops treated group **(Group-I)** the mean value of in VAS observed at baseline (0.day),before treatment (BT)) was 20.83 and it was 0.52 while after treatment at 28th day (AT) **(p-value=<0.001).**

In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value of in VAS observed at baseline (0.day),before treatment (BT))was 25.83 and it was 0.48 while after treatment at 28th day (AT) **(p-value=<0.001).**

The mean value in reduction of symptoms in test intervention (DY Drops) is comparable toconventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant**(p-value=<0.001).(Tables-27,28,29,and 33) (Graphs-11 ,12 and 13)**

GRAPH-11.Mean values of Changes in 'Struck lids ' Before and After treatment (n=138)

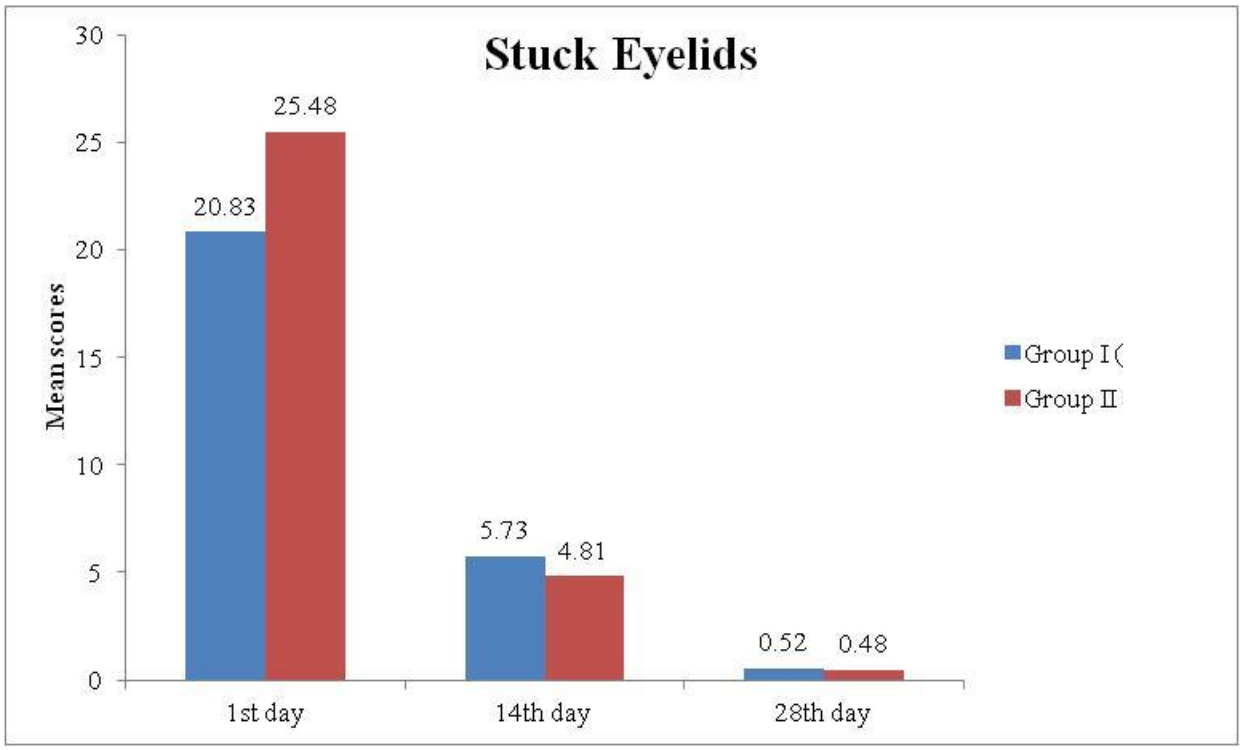


Table-27. Mean values of Changes in 'Stuck lids' Before and After treatment in Group-I (n =67)

Stuck eyelids	Mean	N	Std. Deviation	Std. Error Mean
1st day	20.83	48	26.962	3.892
14th day	5.73	48	12.882	1.859
28th day	.52	48	3.608	.521

Stuck eyelids	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	15.104	25.651	3.702	7.656	22.552	4.080	47	<0.001
1st day - 28th day	20.313	26.626	3.843	12.581	28.044	5.285	47	<0.001

Table-28. Mean values of Changes in 'Stuck lids' Before and After treatment in Group-II (n =71)

Stuck eyelids	Mean	N	Std. Deviation	Std. Error Mean
1st day	25.48	52	28.650	3.973
14th day	4.81	52	12.166	1.687
28th day	.48	52	3.467	.481

Stuck eyelids	Paired Differences					t-value	df	p-value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower Bound	Upper Bound			
1st day - 14th day	20.673	25.592	3.549	13.548	27.798	5.825	51	<0.001
1st day - 28th day	25.000	28.440	3.944	17.082	32.918	6.339	51	<0.001

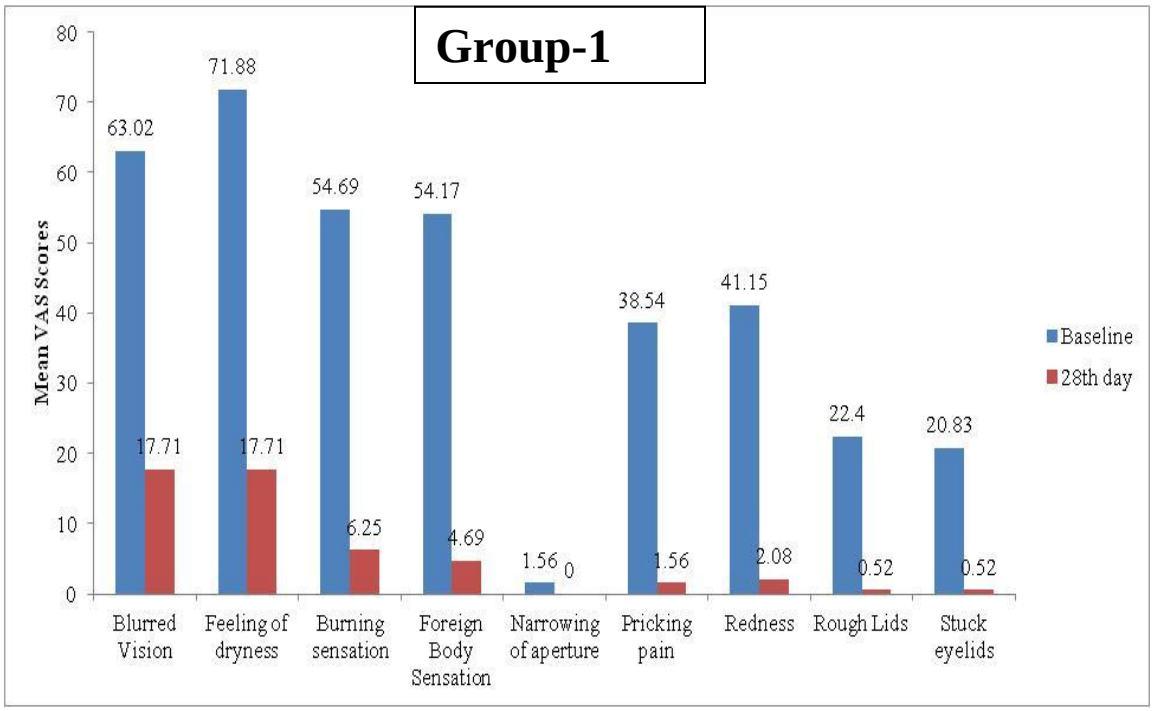
Table-29. Mean values of Changes in 'Stuck lids' Before and After treatment in Group-I and Group-II (n =138)

Independent sample t-test

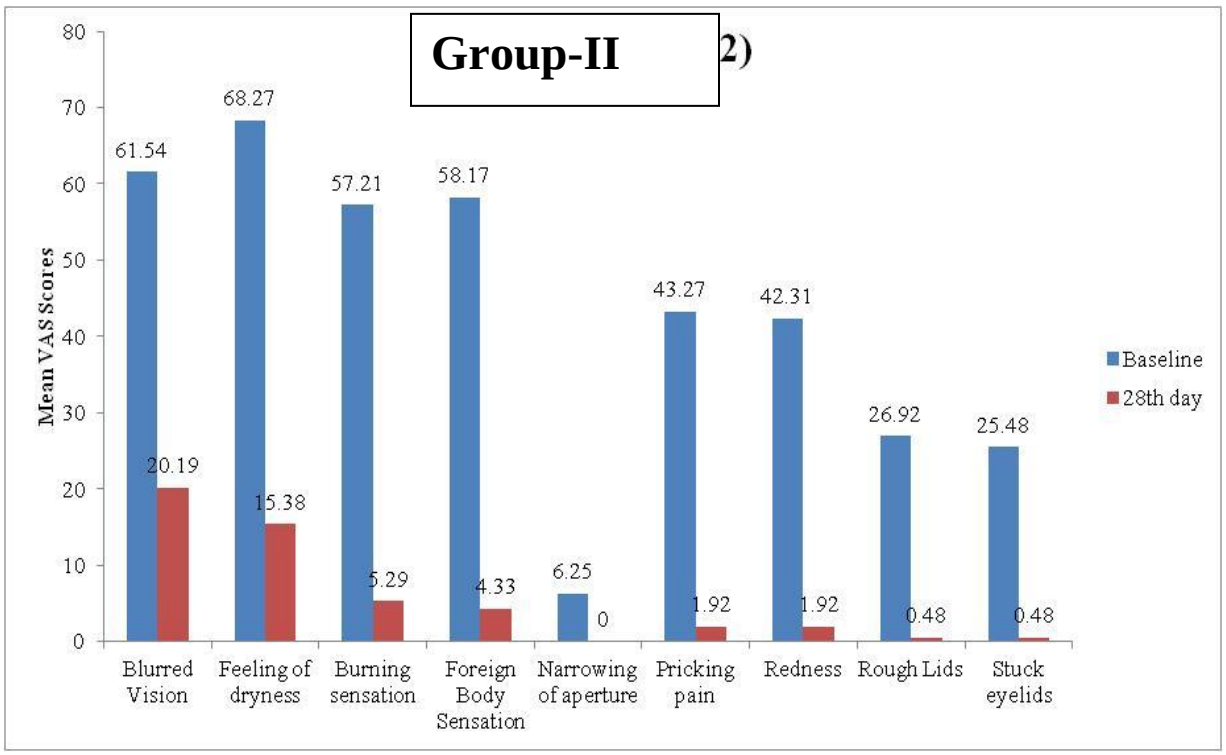
Stuck eyelids	Group	N	Mean	Std. Deviation	Std. Error Mean
Diff of Baseline and 28th day	**1**	48	20.31	26.626	3.843
	2	52	25.00	28.440	3.944

Stuck eyelids	t-test for Equality of Means						
	t-value	df	p-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower Bound	Upper Bound
Diff of Baseline and 28th day	.849	98	.398	-4.688	5.521	-15.644	6.269

GRAPH-12 .Mean Values of Clinical features in Group-1 before and after Treatment (n=67)



GRAPH-13.Mean Values of Clinical features in Group-II before and after Treatment (n=71)



B. Objective Parameters/ Tests: The clinical assessment comprises out come in changes in the Schirmer-I, Test Tear Film Break up Time(TUBT)and changes in Rose Bengal Staining before and after employing test intervention .

1. Shimmers I Test(in mm.) : The mean value changes of Left eye and Right eye at base line and 28th day are as under :

1.1. Shimmers I Test(in mm.) -Left Eye: In DY drops treated group **(Group-I)** the mean value of Shimmers I Test(in mm.)**left eye** observed at baseline (0.day),before treatment (BT) was 5.58 and it was 14.16 while after treatment at 28th day (AT) **(p-value=<0.001).**

In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value of Shimmers I Test(in mm.) **left eye** observed at baseline (0.day),before treatment (BT))was 5.90 and it was 14.74 while after treatment at 28th day (AT) **(p-value=<0.001).**

The mean value in reduction of symptoms in test intervention (DY Drops) is comparable toconventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant**(p-value=<0.001).(Tables-30,31,32, 33 and 34) (Graphs-14)**

1.2. Shimmers I Test(in mm.) -Right Eye: In DY drops treated group **(Group-I)** the mean value of Shimmers I Test(in mm.)**right eye** observed at baseline (0.day),before treatment (BT) was 5.66 and it was 13.34 while after treatment at 28th day (AT) **(p-value=<0.001).**

In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value of Shimmers I Test(in mm.) **right eye** observed at baseline (0.day),before treatment (BT))was 5.88 and it was 13.64 while after treatment at 28th day (AT) **(p-value=<0.001).**

The mean value in reduction of symptoms in test intervention (DY Drops) is comparable toconventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant **(p-value=<0.001).(Tables-30,31,32, 33 and 34) (Graphs-14)**

GRAPH-14 . Mean Values of Shimmers I test before and after Treatment (n=138)

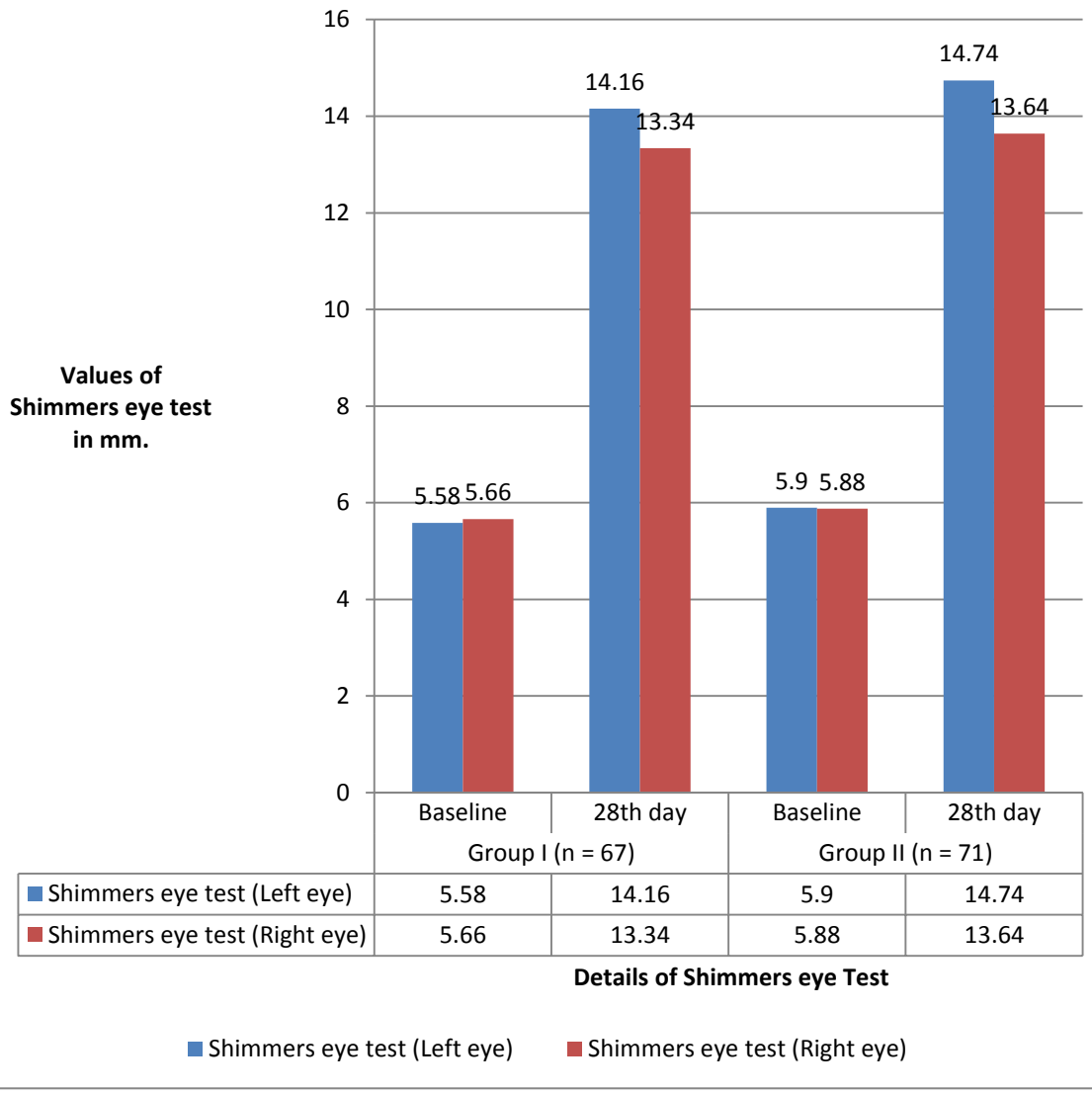


Table-30. Mean values of Changes in 'Shimmers I test' Before and After treatment in Group-I (n =67)

		Mean	N	Std. Deviation	Std. Error Mean
Left Eye	1st day	5.58	50	2.322	.328
	14th day	9.14	50	3.077	.435
	28th day	14.16	50	4.372	.618
Right Eye	1st day	5.66	50	2.471	.349
	14th day	8.98	50	3.191	.451
	28th day	13.34	50	3.967	.561

		Paired Differences					t-value	df	p-value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower Bound	Upper Bound			
Left Eye	1st day – 14th day	-3.560	1.971	.279	-4.120	-3.000	12.773	49	<0.001
	1st day – 28th day	-8.580	3.357	.475	-9.534	-7.626	18.073	49	<0.001
Right Eye	1st day – 14th day	-3.320	1.856	.263	-3.848	-2.792	12.645	49	<0.001
	1st day – 28th day	-7.680	2.744	.388	-8.460	-6.900	19.793	49	<0.001

Table-31. Mean values of Changes in 'Shimmers I test' Before and After treatment in Group-II (n =71)

		Mean	N	Std. Deviation	Std. Error Mean
Left Eye	1st day	5.90	50	2.315	.327
	14th day	9.70	50	3.072	.434
	28th day	14.74	50	4.024	.569
Right eye	1st day	5.88	50	2.336	.330
	14th day	9.38	50	2.641	.373
	28th day	13.64	50	3.837	.543

		Paired Differences					t-value	df	p-value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower Bound	Upper Bound			
Left Eye	**1st day – 14th day**	-3.800	1.654	.234	-4.270	-3.330	-16.249	49	<0.001
	1st day – 28th day	-8.840	3.026	.428	-9.700	-7.980	-20.656	49	<0.001
Right Eye	**1st day – 14th day**	-3.500	1.403	.198	-3.899	-3.101	-17.635	49	<0.001
	1st day – 28th day	-7.760	2.737	.387	-8.538	-6.982	-20.047	49	<0.001

Table-32. Mean values of Changes in 'Shimmers I test' Before and After treatment in Group-I and GroupII (n =138)

Independent sample t-test

Shimmers test	group	N	Mean	Std. Deviation	Std. Error Mean
Diff of Baseline and 28th day (Left eye)	**1**	50	-8.58	3.357	.475
	2	50	-8.84	3.026	.428
Diff of Baseline and 28th day (Right eye)	**1**	50	-7.68	2.744	.388
	2	50	-7.76	2.737	.387

	t-test for Equality of Means						
	t-value	df	p-value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower Bound	Upper Bound
Diff of Baseline and 28th day (Left eye)	.407	98	.685	.260	.639	-1.008	1.528
Diff of Baseline and 28th day (Right eye)	.146	98	.884	.080	.548	-1.008	1.168

TABLE -33 . Mean Values of Shimmers I test before and after Treatment (n=138)

Details of the Eye	Group I (n = 67)		Group II (n = 71)	
	Baseline	28th day	Baseline	28th day
Shimmers I test (Left eye)	5.58 mm.	14.16 mm	5.90 mm	14.74 mm
Shimmers I test (Right eye)	5.66 mm.	13.34 mm	5.88 mm	13.64 mm

Table-34. Mean values of Changes in 'Clinical features and Shimmers I test' Before and After treatment in Group-I and Group II (n =138)

S.No	Clinical features and Shimmers I test	Mean VAS Scores and changes in Shimmers I test p-value =<0.001			
		Group I (n = 67)		Group II (n = 71)	
		Baseline	28th day	Baseline	28th day
1.	Blurred Vision	63.02	17.71	61.54	20.19
2.	Feeling of dryness	71.88	17.71	68.27	15.38
3.	Burning sensation	54.69	6.25	57.21	5.29
4.	Foreign Body Sensation	54.17	4.69	58.17	4.33
5.	Narrowing of aperture	1.56	0.00	6.25	0.00
6.	Pricking pain	38.54	1.56	43.27	1.92
7.	Redness	41.15	2.08	42.31	1.92
8.	Rough Lids	22.40	0.52	26.92	0.48
9.	Stuck eyelids	20.83	0.52	25.48	0.48
10.	Shimmers I test (Left eye) in mm.	5.58	14.16	5.90	14.74
11.	Shimmers I test (Right eye)) in mm.	5.66	13.34	5.88	13.64

2. Tear film break-up Time (TUBT) in Seconds: The mean value changes of Left eye and Right eye at base line and 28th day are as under:

2.1. Tear film break-up Time (TUBT) in Seconds -Left Eye: In DY drops treated group **(Group-I)** the mean value of TUBT Test (in sec.)**left eye** observed at baseline (0.day),before treatment (BT) was 8.97 and it was 14.43 while after treatment at 28th day (AT) **(p-value=<0.001).**

In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value TUBT Test (in sec.)**left eye** observed at baseline (0.day),before treatment (BT))was 8.74 and it was 15.32 while after treatment at 28th day (AT) **(p-value=<0.001).**

The mean value in reduction of symptoms in test intervention (DY Drops) is comparable toconventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant**(p-value=<0.001).(Table-35) (Graphs-15)**

2.2. Tear film break-up Time (TUBT) in Seconds -Right Eye: In DY drops treated group **(Group-I)** the mean value of TUBT Test(in sec.)**right eye** observed at baseline (0.day),before treatment (BT) was 8.52and it was 15.87 while after treatment at 28th day (AT) **(p-value=<0.001).**

In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** the mean value of TUBT Test(in sec.) **right eye** observed at baseline (0.day),before treatment (BT))was 7.92 and it was 14.45 while after treatment at 28th day (AT) **(p-value=<0.001).**

The mean value in reduction of symptoms in test intervention (DY Drops) is comparable toconventional control tear supplement Carboxy methyl cellulose) revealing the efficacy of DY eye drops which is statistically significant **(p-value=<0.001).(Tables-35) (Graphs-15)**

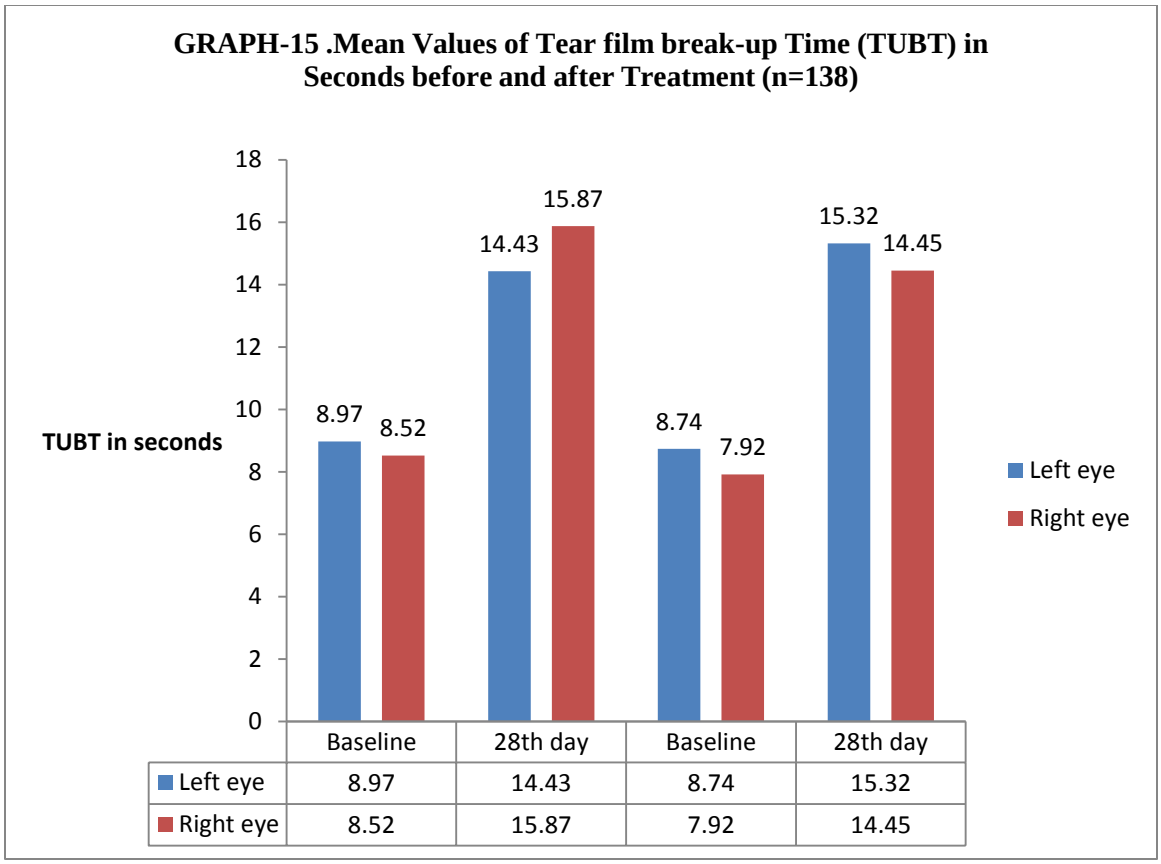


TABLE -35. Mean Values of Tear film break-up Time (TUBT) in Seconds before and after Treatment (n=138)

Tear film break-up time(TUBT) in Seconds	Group I (n = 67)		Group II (n = 71)	
	Baseline	28th day	Baseline	28th day
Left eye	8.97 sec.	14. 43sec.	8.74 sec.	15.32. sec
Right eye	8.52 sec.	15.87 sec.	7.92 sec.	14.45 sec

3. Rose Bengal staining (Oxford scheme of scoring): The mean value changes of Left eye and Right eye at base line and 28th day are as under:

3.1 Group-1-Rose Bengal staining (Oxford scheme of scoring)

Left Eye: In DY drops treated group **(Group-I)** at baseline (0.day),before treatment (BT), No staining was observed in 0 subjects, Mild staining in 38, Moderate staining in 21 , Moderately Severe staining in 7 and Intense staining was noticed in 2 participants . While after treatment at 28th day (AT) No staining was observed in 51 subjects, Mild staining in 10, Moderate staining in 5 , Moderately Severe staining in 0 and Intense staining was noticed in 1 participants indicating significant improvement in corneal wetting. **(p-value=<0.001).**

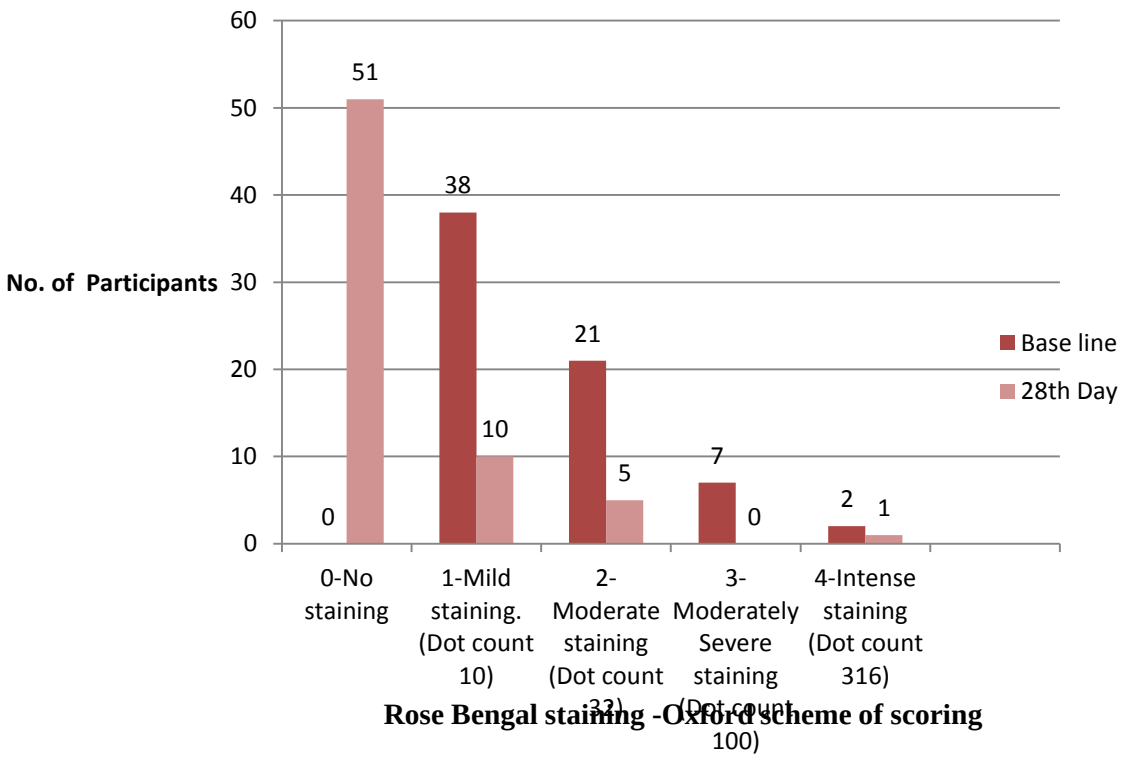
Right Eye: In DY drops treated group **(Group-I)** at baseline (0.day),before treatment (BT), No staining was observed in 0 subjects, Mild staining in 36, Moderate staining in 24 , Moderately Severe staining in 6 and Intense staining was noticed in 1 participants . While after treatment at 28th day (AT) No staining was observed in 53 subjects, Mild staining in 5, Moderate staining in 6 , Moderately Severe staining in 3 and Intense staining was noticed in 0 participants indicating significant improvement in corneal wetting. **(p-value=<0.001). (Tables-37) (Graphs-16 and 17)**

3.2 Group-II-Rose Bengal staining (Oxford scheme of scoring)

Left Eye: In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** at baseline (0.day),before treatment (BT), No staining was observed in 0 subjects, Mild staining in 28, Moderate staining in 39 , Moderately Severe staining in 3 and Intense staining was noticed in 1 participants While after treatment at 28th day (AT) No staining was observed in 58 subjects, Mild staining in 11, Moderate staining in2 , Moderately Severe staining in 0 and Intense staining was noticed in 0 participants indicating significant improvement in corneal wetting. **(p-value=<0.001).**

Right Eye: In conventional control tear supplement Carboxy methyl cellulose) treated group **(Group-II)** at baseline (0.day),before treatment (BT), No staining was observed in 0 subjects, Mild staining in 32, Moderate staining in 36 , Moderately Severe staining in 2and Intense staining was noticed in 1 participants . While after treatment at 28th day (AT) No staining was observed in 61 subjects, Mild staining in 9, Moderate staining in 0 , Moderately Severe staining in 1 and Intense staining was noticed in 0 participants indicating significant improvement in corneal wetting. **(p-value=<0.001).** **(Tables-37) (Graphs-18 and 19)**

GRAPH-16 .Change in Rose Bengal staining (Oxford scheme of scoring) before and after Treatment in LEFT EYE of Treatment Group -I (n=67)



GRAPH-16 .Change in Rose Bengal staining (Oxford scheme of scoring) before and after Treatment in LEFT EYE of Treatment Group -I (n=67)

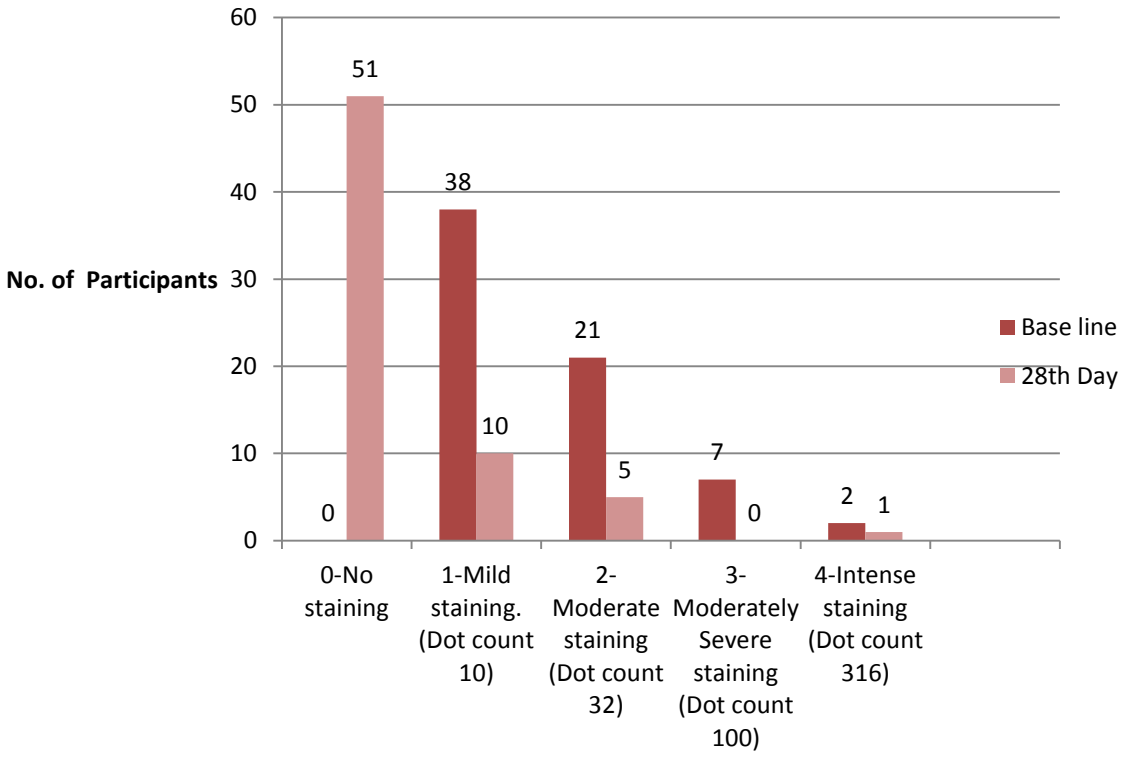


TABLE-36 . Change in Rose Bengal staining (Oxford scheme of scoring) before and after Treatment in Treated Group –I (n=67)

Oxford scheme of scoring	Group I (n = 67)	
	Baseline	28th day
Left eye		
0-No staining	0	51
1-Mild staining. (Dot count 10)	38	10
2- Moderate staining (Dot count 32)	21	5
3-Moderately Severe staining (Dot count 100)	7	0
4-Intense staining (Dot count 316)	2	1
Right eye		
0-No staining	0	53
1-Mild staining. (Dot count 10)	36	5
3-Moderately Severe staining (Dot count 100)	24	6
3-Moderately Severe staining (Dot count 100)	6	3
4-Intense staining (Dot count 316)	1	0

TABLE- 37 . Change in Rose Bengal staining (Oxford scheme of scoring) before and after Treatment in Standard control Group -II(n=71)

Oxford scheme of scoring	Group II (n =71)	
	Baseline	**28th day**
Left eye		
0-No staining	0	58
1-Mild staining. (Dot count 10)	28	11
2- Moderate staining (Dot count 32)	39	2
3-Moderately Severe staining (Dot count 100)	3	0
4-Intense staining (Dot count 316)	1	0
Right eye		
0-No staining	0	61
1-Mild staining. (Dot count 10)	32	9
3-Moderately Severe staining (Dot count 100)	36	0
3-Moderately Severe staining (Dot count 100)	2	1
4-Intense staining (Dot count 316)	1	0

C. Drug compliance and adherence to dose schedules: The assessing for drug compliance was done through inquiry and also collecting the empty eye drop containers at 14^{th} and 28^{th} days and confirmed .

D. ADRs(Adverse Drug Reaction) and AEs(Adverse Events) Reporting: No ADRs(Adverse Drug Reaction) or AEs(Adverse Events) were Reported confirmed by inquiry and clinical examination.

Overall Assessment: :The analysis of various parameters revealed statistically significant relief of subjective parameters and clinical symptoms of dry eye such as Blurred Vision, Feeling of dryness, Burning sensation, Foreign Body Sensation, Narrowing of aperture, Pricking pain, Redness, Rough Lids, Stuck eyelids which were assessed using Visual Analogue Scale (VAS)**(p-value=<0.001).** Alongside this, the objective assessment of Tear film break-up time(TUBT),Schirmer-I test, Rose Bengal staining performed at baseline(0 –day and 28^{th} day has shown remarkable progress in terms of improving wetting of ocular surface and restoration of components of tear film functions. **(p-value=<0.001).** Furthermore it is worth noting that the formulation is well tolerated without ADRs And AEs**.(Graph -12,13 and 14 ;Tables.34 and 35)**

Chapter- 6
Discussion

Chapter-6: Discussion

The study revealed statistically significant relief of subjective parameters and clinical symptoms of dry eye such as Blurred Vision, Feeling of dryness, Burning sensation, Foreign Body Sensation, Narrowing of aperture, Pricking pain, Redness, Rough Lids, Stuck eyelids which were assessed using Visual Analogue Scale (VAS)**(p-value=<0.001).** Adding to this the objective assessment of Tear film break-up time(TUBT),Schirmer-I test, Rose Bengal staining performed at baseline(0 –day and 28th day has shown remarkable progress in terms of improving wetting of ocular surface and restoration of components of tear film functions. **(p-value=<0.001).** Besides this, the eye drops are well tolerated without complications, ADRs and AEs.

The under mentioned discussion substantiate the role of the current Ayurvedic trial intervention -'DY Eye drops' prepared with Daruharidra (*Berberis aristata* DC.) & Yastimadhu (*Glyeyrrhiza glabra* Linn.) in the effective management of Dry Eye syndrome.

Scientific Rational: Effective management of Dry eye syndrome calls for all inclusive approach to address and tackle underlying pathology ,precipitating and management of symptoms .It is pivotal to address all these attributes and factors while developing and validating therapies. The efficacy of eye drops could be defensible with following substantiating elucidation of underlying factors, unmet needs and gaps in conventional management approach for dry eye, need for development of comprehensive management approach for dry eye and scientific basis for formulation and evolving inclusive therapeutic strategies taking leads from Ayurvedic literatures and contemporary studies .

1. Role Underlying contributory factors: Dry Eye Syndrome is a leading cause of ocular discomfort affecting millions of people. Dry Eye conditions are a spectrum of disorders with varied etiology ranging from mild eyestrain to very severe dry eyes associated with visual complications. Dry eye is a condition produced by the inadequate interrelation between lachrymal film and ocular surface epithelium, and is caused by quantitative and qualitative deficits in one or both of them. Ocular surface conditions may

result from the abnormalities in one or more of the tear film components, ocular or systemic diseases, various drugs and even environment factors. Oxidative stress is involved in many surface ocular diseases including dry eye syndrome (DES).

2.Unmet needs and gaps in conventional management approach for Dry eye syndrome: Contemporary management strategies for Dry eye syndrome pose certain limitations. Management strategies of DES include mainly supplementation of tear preferably substitutes containing methylcellulose or carboxy-methylcellulose or identical substances which are viscous in nature. Preservatives used in formulations are known to cause dry eyes. All these drugs do not have any effect on basic path physiology and they provide only symptomatic relief .The tear stimulants such as cholinergic drugs increases the tear production from lacrimal gland by stimulating secretions, but not been used in clinical practice. Tear Preservation can be done by occluding the puncta or minimizing evaporation, but is useful as short-term measure to assess the effect of occluding puncta before resorting to permanent measures. All these drugs do not have any effect on basic path physiology and they provide only symptomatic relief Further Topical antibiotics and corticosteroids are sometimes used to treat secondary infections and inflammation. But discontinuation of antibiotics, steroids and all preservative-containing eye drops is mandatory for relief of symptoms and progressively improving the tear film and ocular surface.

3. Need for development an comprehensive management approach for Dry Eye Syndrome: In view of the above, there is an urgent need to evolve safe and effective management approach to tackle symptoms, infections and underlying pathology. This calls for development of ideal pharmaceutical dosage form addressing the issues of symptom management alongside other contributory factors of underlying pathology such as control and management of ocular infections ,restoration of surface ocular health by offering antioxidants to prevent oxidative damage.

4. Restoration of protective functions of tear film comparable to *tarpaka-kapha* : Tear film comparable to *tarpaka-kapha* plays a pivotal role in maintaining the surface environment and lubricating the eye**.** As enumerated in Ayurvedic texts *Kapha* is responsible for *sanigdhatwa*(lubrication) *sthiratwa* (structural and functional integrity of

body systems) by virtue of its qualities like *gurutwa* and *snigdwatva*. *Tarpaka-kapha*, one among the five varieties of *Kapha*, situated in head (*siras*)is responsible for the integrity of sense organs (*aksha-tarpana*). According to *Dalhana* the term *aksha* refers to sense organs such as eye.Collective function tear film components can be correlated with the function of the *tarpa-kakapha*

The pre-corneal tear film has three layers, the outer lipid layer, the middle aqueous layer and the inner mucin layer, each has its own role to play viz. to retard the evaporation of aqueous layer, to increase surface tension so that the film is stable, to lubricate the eye lids, to supply atmospheric oxygen to corneal epithelium and it has anti-bacterial enzymes; laysozyme and lactoferin**.** Ayurvedic literatures recount the *tarpaka-kapha as* the essential factor attributed with functions and protective role identical to that of tear film. The intervention comprising of *Daruharidra* (*Berberis aristata* DC.) & *Yastimadhu* (*Glyeyrrhiza glabra* Linn. poses a crucial role in restoring tear functions by virtue of its inherent protective immunological preventive ,promotive attributes such as anti-oxidant, (*netrya*,,*chakushya –rasayana)* anti-microbial(*krimighna*), anti-inflammatory (*netra sotha hara*), *netraruja-hara* (analgesic ophthalmic action),

4. Scientific basis drawn from Ayurvedic textual leads and contemporary science and ophthalmology for developing Ayurvedic intervention : The standardized and quality assured eye drops rationally developed form from Ayurvedic ophthalmic plant drugs *Daruharidra* (*Berberis aristata* DC.) & *Yastimadhu* (*Glyeyrrhiza glabra* Linn. with proven anti allergic, analgesic anti-inflammatory , anti-microbial and anti-oxidant potential in surface ocular conditions which could offer safe, effective and comprehensive management .Further the pharmacological actions recounted attributed in Ayurvedic texts such as *caksusya* (conducive to vision), *netrya* (conducive to adnexa of eye), *netraruja-hara* (analgesic ophthalmic action), *netra-sodhahara*(anti-inflammatory action) *netrakanduhara* (anti allergic action), *vrana-ropana*(wound healing effect) to *Daruharidra* (*Berberis aristata* DC.) & *Yastimadhu* (*Glyeyrrhiza glabra* Linn.) which are contributory to comprehensive- management of dry eye disease by restoring the functions of tear film comparable to functional attribute –*tarpaka kapha*.

5. Tangible scientific evidence in evolving Inclusive therapeutic strategies: The above leads and current pre-clinical studies on antimicrobial activity ,antioxidant potential and ocular safety and toxicity studies made the clinical evidence more tangible and scientific as reflected under.

A. In vitro anti-oxidant studies of eye drops as protective role of antioxidant supplementation in the maintenance ocular surface health and restoration tear functions (*tarpaka kapha*): Oxidative stress in the cornea influenced by several environmental factors such as air pollution, radiation, chemicals etc. leads to changes in corneal optical properties and decrease in visual acuity or even vision loss. The antioxidant agents help in suppressing the damages due to oxidative stress hand assist in restoring the corneal health The surface lesions and corneal diseases, associated with oxidative stress leads to corneal aging, b corneal inflammation In acute corneal inflammation the Reactive oxygen species (ROS) are highly involved. Studies on the oxidative reactions in tears of patients with dry eye disease confirmed a marked increase of inflammatory activity in the tear film of patients suffering from dry eye. These reactions lead to severe damage of the eye. Free radicals and inflammation may be involved in the pathogenesis or in the self-propagation of the dry eye disease**.** The antioxidant therapy with superoxide dismutase and dimethyl thiourea are employed for the healing of corneal ulcers evoked by sodium hydroxide. Topical antioxidant therapy found effective in reducing the inflammatory corneal reaction

The anterior eye segment and mainly the cornea are directly affected by the hazards from potential oxidative damage evoked by air pollution, radiation, chemicals as evident by proven role of oxidative stress in the pathogenesis surface ocular disease. Further certain scientific studies have demonstrated beneficial effects and protective role of topical and oral antioxidants in the management of surface lesions like dry eye disease. Along with measures such as tear preservation, use of tear stimulants, tear substitutes and control of infection, the advocacy of topical and oral antioxidant agents also forms an important component in the management of dry eye syndrome (DES).

Owing to its importance, the biochemical assessment of antioxidant potential of the Ayurvedic herbal eye drops formulated for dry eye syndrome was performed.. The study encompasses biochemical antioxidant assays viz. Nitric oxide radical scavenging assay, ABTS radical scavenging assay, DPPH radical scavenging assay and Ferric reducing antioxidant potential (FRAP) assay. These studies revealed significant antioxidant potential of herbal eye drops, possibly compliment to the management of dry eye syndrome and marinating the surface ocular health.

B. Antimicrobial assays of eye drops as an agent for management of infection-A Pivotal aspect in Dry Eye Disease : Dry eye disease commonly occurs after an episode of viral kerato-conjunctivitis or severe acute or sub-acute conjunctivitis. These diseases may lead to loss of goblet cells from the conjunctival epithelium and release of inflammatory cytokines. Patients usually complain of persistent symptoms and continue to be treated for the original condition.

This treatment is not only inappropriate, it may also be toxic; whereas they are actually suffering from the vicious cycle of secondary tear film alterations. The study encompasses antibacterial assays for determination of Minimum Inhibitory Concentration (MIC) using pathogenic strains of bacteria; Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhinurium, Escherichia coli, Klebsiella pneumonia as test organisms and screening of antifungal activity of eye drops against *Aspergillus fumigatus* and *Candida albicans*. The studies revealed notable anti-bacterial and anti-fungal activity against selected microbial strains, conceivably compliment to the management of dry eye syndrome.

Further the anti-microbial property certainly compliment to symptom management and mitigation of underlying pathology linked with tear component deficiency. The eye drops formulated rationally with Ayurvedic herbal ingredients may contribute significantly by offering comprehensive management for dry eye syndrome.

C. Pre-clinical studies for ensuring quality assurance and ocular safety and toxicity : The eye drops formulated was developed and standardized in compliance with guidelines as applicable to ensure quality . Further Acute Eye Irritation tests and ocular toxicity studies revealed that the eye drops did not cause irritation to ocular mucous membrane of eyes of rabbits such as opacity ulceration of cornea; congestion, swelling, moderate circum-corneal hyperaemia; injection, hemorrhage, gross destruction of iris; redness and other signs of inflammation of palpebral and bulbar conjunctivae; chemosis of lids etc.and no evident signs of toxicity were observed. Further, no clinical signs of mortality and change in body weight were noticed during the observation period of 72 hours of post application, was found to be practically nonirritant to the eyes .

D. Drug compliance and tolerability The assessing for drug compliance was done through inquiry and also collecting the empty eye drop containers at 14^{th} and 28^{th} days and confirmed .Further No ADRs(Adverse Drug Reaction) or AEs(Adverse Events) were reported confirmed by inquiry and clinical examination.

It is evident from pre-clinical and clinical studies that the drug intervention under the study comprising of *Daruharidra* (*Berberis aristata* DC.) & *Yastimadhu* (*Glyeyrrhiza glabra* Linn. is safe, effective and well tolerated.

Chapter-7
Summary

Chapter-7: Summary

Despite progress in determining the etiology and pathogenesis of dry eye syndrome, current knowledge remains inadequate, and no preventive strategies have been found. The present-day management strategy of dry eye syndrome though clinically effective, poses certain limitations. Preservatives used in formulations are known to cause dry eyes. The tear stimulants such as cholinergic drugs increases the tear production from lacrimal gland by stimulating secretions, but not been used in clinical practice. All these drugs do not have any effect on basic path physiology and they provide only symptomatic relief Topical antibiotics and corticosteroids are sometimes used to treat secondary infections and inflammation. But discontinuation of antibiotics, steroids and all preservative-containing eye drops is mandatory for relief of symptoms and progressively improving the tear film and ocular surface. Moreover, the most common therapy for dry eye syndrome, artificial tears, provides only temporary and incomplete symptomatic relief. Hence, identification of modifiable risk factors for dry eye syndrome may suggest avenues for investigation of novel preventive and treatment measures

Considering this , it is becomes essential to translate some promising leads for management of dry eye syndrome from Ayurvedic literatures into, safe, effective and quality assured dosage forms to improve patient's compliance. In addition to the management of symptoms related to deficiency of tear components, prevention of damage due oxidative stress, arrest of further progress and control of infection also forms an vital component in dry eye disease. The close relationship between ocular surface epithelia and the pre-ocular tear film ensures ocular surface health. As such, dysfunctional protective elements that lead to ocular surface and tear disorders are heterogeneous, effective therapeutic strategies are the need of hour to tackle tear disorders attributed with diverse factors.

Tear film comparable to *tarpaka-kapha* plays a pivotal role in maintaining the surface environment and lubricating the eye**.** The pre-corneal tear film has three layers, the outer lipid layer, the middle aqueous layer and the inner mucin layer, each has its own role to play viz. to retard the evaporation of aqueous layer, to increase surface tension so that the

film is stable, to lubricate the eye lids, to supply atmospheric oxygen to corneal epithelium and it has anti-bacterial enzymes; laysozyme and lactoferin**.** The disturbance in the functional components of *tarpaka-kapha* or pre-corneal layers leads to dry inflammation of the eye or *Shushkakshipaka*,

Ayurvedic literatures recount potential ophthalmic drugs for the management of surface inflammatory conditions of eye such as*sushkashipaka* or *parisushkanetra*a comparable with dry eye syndrome(DES) or Kerato-conjunctivitis Sicca(KCS) . These plants are attributed with Pharmacological actions such as *caksusya* (conducive to vision), *netrya* (conducive to adnexa of eye), *netraruja-hara* (analgesic ophthalmic action), *netra-sodhahara*(anti-inflammatory action) *netrakanduhara* (anti allergic action), *vrana-ropana*(wound healing effect) supported by scientific evidences

Further scientific experimental studies also revealed antioxidant, anti-bacterial, antifungal, antioxidant which are contributory to comprehensive- management of dry eye disease by restoring the functions of tear film comparable to functional attribute – *tarpaka kapha*.

With this rationale and background, an Ayurvedic eye drops was developed for dry eye syndrome (DES) systematically following appropriate methods and parameters right from quality assurance of ingredients, formulation of standard operation procedures (SoPs) and also complying to the quality and safety standards of finished product for ophthalmic preparation as specified in Ayurvedic Pharmacopeia of India and Indian Pharmacopeia. (N. Srikanth, Arjun Singh,,Sharad D. Pawar ,S. N. Murthy and R.R. Padmavar. Development and Standardization of An Ayurvedic Herbal Eye Drops for Dry Eye Syndrome, World Journal of Pharmaceutical Research, 06/2015; 4(6) 1034-1041.)

The ocular toxicity studies of standardized herbal eye drops revealed its safety on topical ophthalmic use. (N. Srikanth, Sharad D. Pawar, Arjun Singh, S. N. Murthy and R.R. Padmavar. Ocular Safety and Toxicity Studies of An Ayurvedic Herbal Eye Drop for Dry Eye Syndrome, European Journal of Biomedical and Pharmaceutical sciences, 06/2015; 2(3):679-687)

Further the antioxidant and anti-microbial property certainly contributes to effective symptom management and extenuation of basic pathology linked with tears component deficiency. (N. Srikanth, Sharad D. Pawar, Arjun Singh, S. N. Murthy and R.R. Padmavar..IN VITRO BIOCHEMICAL ASSESSMENT OF ANTIOXIDANT POTENTIAL OF AN AYURVEDIC HERBAL EYE DROPS FOR DRY EYE SYNDROME, European Journal of Pharmaceutical and Medical Research.2015,2(6), 261-266; N. Srikanth, Sharad D. Pawar, Arjun Singh, S. N.Murthy and R.R. Padmavar. ANTIMICROBIAL ASSAYS OF AN AYURVEDIC HERBAL EYE DROPS FOR DRY EYE SYNDROME, World Journal of Pharmacy and Pharmaceutical Sciences (Impact Factor: 5.21). 12/2015; Volume 4, Issue 12, 711-721)

Systematic and well-designed clinical studies study revealed statistically significant relief of subjective parameters and clinical symptoms of dry eye such as Blurred Vision, Feeling of dryness, Burning sensation, Foreign Body Sensation, Narrowing of aperture, Pricking pain, Redness, Rough Lids, Stuck eyelids .Adding to this the objective assessment of Tear film break-up time(TUBT),Schirmer-I test, Rose Bengal staining performed at baseline (0 –day) and 28^{th} day has shown remarkable progress in terms of improving wetting of ocular surface and restoration of components of tear film functions. Besides this, the eye drops are well tolerated without complications, ADRs and AEs.

The eye drops containing *Daruharidra* (*Berberis aristata* DC.) & *Yastimadhu* (*Glyeyrrhiza glabra* Linn) developed rationally taking potential leads from codified Ayurvedic texts probably contribute by offering comprehensive management for dry eye syndrome .The scientific pre-clinical and clinical studies generated a perceptible evidence and revealed that the eye drop safe , well tolerable and clinically effective comparable to conventional control drug tear supplement-Carboxy methyl cellulose.

Chapter-8
Conclusion

Chapter-8: Conclusion

1. Ayurvedic literatures recount Dry Eye Syndrome (DES) or 'kerato-conjunctivitis-sicca'(KCS) as *Shushkakshipaka, Parishuskha-netra, Ativishuskha-netra, Asrusravarahita-netra* and *Asnigdha-netra* indicative of dryness of eye due to deficiency in tear film components.
2. *Tarpaka-kapha*, one among the five varieties of *Kapha*, situated in head (*siras*)is responsible for the integrity of sense organs (*aksha-tarpana*). According to *Dalhana* the term *aksha* refers to sense organs such as eye. Collective function tear film components can be correlated with the function of the *tarpa-kakapha*
3. The disturbance in the functional components of *tarpaka-kapha* or pre-corneal layers leads to dry inflammation of the eye or *Shushkakshipaka,*
4. The most common therapy for dry eye syndrome, artificial tears, and other conventional approaches provide only temporary and incomplete symptomatic relief. Hence, identification of modifiable risk factors for dry eye syndrome may suggest avenues for investigation of novel preventive and treatment measures
5. Ayurvedic literatures enumerate potential ophthalmic drugs for the management of surface inflammatory conditions of eye such as *Daruharidra* (*Berberis aristata* DC.) & *Yastimadhu* (*Glyeyrrhiza glabra* Linn)..These plants are attributed with Pharmacological actions such as *caksusya* (conducive to vision), *netrya* (conducive to adnexa of eye), *netraruja-hara* (analgesic ophthalmic action), *netra-sodhahara* (anti-inflammatory action) *netra-kanduhara* (anti allergic action), *vrana-ropana* (wound healing effect).Further scientific experimental studies also revealed antioxidant, anti-bacterial ,antifungal, antioxidant which are contributory to comprehensive- management of dry eye disease by restoring the functions of tear film comparable to functional attribute –*tarpakakapha*.
6. Baked by this rationale and leads, an Ayurvedic eye drops containing *Daruharidra* (*Berberis aristata* DC.) &*Yastimadhu* (*Glyeyrrhiza glabra* Linn)was developed for dry eye syndrome (DES) systematically following appropriate methods and parameters right from quality assurance of ingredients, formulation of standard operation procedures (SoPs) and also complying to the quality and safety standards of

finished product for ophthalmic preparation as specified in Ayurvedic Pharmacopeia of India and Indian Pharmacopeia.

7. The Eye drops did not cause irritation to ocular mucous membrane of eyes of rabbits such as opacity ulceration of cornea; congestion, swelling, moderate circum corneal hyperaemia; or injection, hemorrhage, gross destruction of iris; redness and other signs of inflammation of palpebral and bulbar conjunctivae; chemosis of lids etc. and no evident signs of toxicity were observed. Further, no clinical signs of mortality and change in body weight were noticed during the observation period of 72 hours post application and the eye drops was found to be practically nonirritant to the eyes of rabbits. The ocular toxicity studies of standardized herbal eye drops revealed its safety on topical ophthalmic use.
8. The antioxidant studies encompass sing biochemical antioxidant assays viz. Nitric oxide radical scavenging assay, ABTS radical scavenging assay, DPPH radical scavenging assay and Ferric reducing antioxidant potential (FRAP) assay revealed significant antioxidant potential of herbal eye drops, possibly compliment to the management of dry eye syndrome and marinating the surface ocular health.
9. The anti-microbial assays including antibacterial assays for determination of Minimum Inhibitory Concentration (MIC) using pathogenic strains of bacteria; *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhinurium*, *Escherichia coli, Klebsiella pneumonia*as test organisms and screening of antifungal activity of eye drops against *Aspergillus fumigatus* and *Candida albicans*. revealed notable anti-bacterial and anti-fungal activity against selected microbial strains, conceivably compliment to the management of dry eye syndrome.
10. The anti-oxidant and anti-microbial attributes of eye drops certainly contributes to effective symptom management and extenuation of basic pathology linked with tears component deficiency.
11. Further Systematic and well-designed clinical study study revealed statistically significant relief of subjective parameters and clinical symptoms of dry eye such as Blurred Vision, Feeling of dryness, Burning sensation, Foreign Body Sensation, Narrowing of aperture, Pricking pain, Redness, Rough Lids, Stuck eyelids .

12. Adding to this the objective assessment of Tear film break-up time(TUBT),Schirmer-I test, Rose Bengal staining performed at baseline (0 –day) and 28th day has shown remarkable progress in terms of improving wetting of ocular surface and restoration of components of tear film functions. Besides this, the eye drops are well tolerated without complications, ADRs and AEs.

13. The eye drops containing *Daruharidra* (*Berberis aristata* DC.) & *Yastimadhu* (*Glyeyrrhiza glabra* Linn.) developed rationally taking potential leads from codified Ayurvedic texts probably contribute by offering comprehensive management for dry eye syndrome .The scientific pre-clinical and clinical studies generated a perceptible evidence and revealed that the eye drop safe , well tolerable and clinically effective comparable to conventional control drug-tear supplement-Carboxy methyl cellulose.

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Annexure-1

Case Report Forms

CLINICAL EVALUATION OF DARUHARIDRA (BERBERIS ARISTATA) - YASTIMADHU (GLYEYRRHIZA GLABRA) EYE DROPS IN THE MANAGEMENT OF DRY EYE SYNDROME (SHUSHKAKSHIPAKA)

CASE REPORT FORM I – SCREENING

BEFORE TREATMENT
(Enter a √ in the appropriate box)

1. Centre name: _____

2. OPD No: _____

3. Name of the Subject : _____

4. Subject S.No: _____

5. Gender: Male (1) ☐ Female (2) ☐

6. Age: _____years

7. Address: _____

_____

8. Group No. Group-I ☐ Group-II ☐

9. EYE TESTS:

- Tear Film break up time _____sec.
- Schirmer's I Test _____mm
- Rose Bengal Staining

_____

_____

10. INCLUSION CRITERIA

	Yes (1)	**No(0)**
1. Subjects of either sex aged between 35 to 70 years	☐	☐
2. Patients presenting with any of the signs and symptoms of Dry eye syndrome viz Feeling of dryness in the eyes, burning sensation, foreign body sensation (Sandy/ Scratchy / itching), pricking pain, Rough lids / mucoid discharge/ mild blepharitis, stuck eyelids, blurred vision,	☐	☐

redness) with

a. Schirmer-I test positive i.e. < 10 mm.
b. Tear film break-up time less than 10 seconds.
c. Rose Bengal staining showing devitalized epithelium of conjunctiva and mucus plaqueson the cornea.

3. Willing and able to participate in the study for 4 weeks. ☐ ☐

11. EXCLUSION CRITERIA

		Yes (1)	**No(0)**
1.	Severe cases of dry eye syndrome with complications like perforated corneal ulcer, Uveitis, Glaucoma etc.	☐	☐
2.	Associated with Inflammatory conditions like acute conjunctivitis etc.	☐	☐
3.	Systemic diseases causing Dry Eye Syndrome.	☐	☐
4.	Pregnant / lactating females	☐	☐
5.	Patients on steroids, oral contraceptive pills, estrogen replacement therapy or any other medication that may adversely affect the outcome of the study	☐	☐
6.	Patients suffering from major systemic illness necessitating long term drug treatment (Rheumatoid arthritis, Psycho-Neuro- Endocrinal disorders, etc.).	☐	☐
7.	Any other condition which the Investigator thinks may jeopardize the study	☐	☐

12. Whether the subject is suitable for enrollment in the study?

Yes (1) ☐ No (0) ☐

A subject is suitable for enrollment in the trial, if points 1 to 3 among the Inclusion Criteria are 'YES' and points 1 to 7 among the Exclusion Criteria are 'NO'

If enrolled: - Subject Enrollment No.:

Date _____ Signature of Investigator_____

CLINICAL EVALUATION OF DARUHARIDRA (BERBERIS ARISTATA) - YASTIMADHU (GLYEYRRHIZA GLABRA) EYE DROPS IN THE MANAGEMENTOF DRY EYE SYNDROME (SHUSHKAKSHIPAKA)

CASE REPORT FORM II A – HISTORY AT BASELINE

(Enter a √ in the appropriate box)

1. Centre Name: _____
2. Date of Induction into the Clinical Trial:_____
3. Expected Date of Completion of the Clinical Trial:_____

PERSONAL IDENTIFICATION

4. Participant's Enrollment ID:_____
5. O.P.D. No: _____
6. Name of the Participant: _____
7. Age:_____
8. Sex: Male (1) ☐ Female (2) ☐
9. Address ----------

   ----

10. Group No. Group-I Group-II

DEMOGRAPHIC PROFILE

11. **Marital status:** Married (1) ☐ Unmarried (2) ☐ Widow(er) (3) ☐

    Divorcee (4) ☐ Any Other (5) ☐

12. **Educational status**: Illiterate (1) ☐ Read & Write (2) ☐

    If (2), specify educational qualification _____

13. **Occupation:**

Desk Work (1) ☐ Field work with physical labour (2) ☐ House wife (3) ☐

If (2), specify _____

14. **Socio-economic status:** Above Poverty line (1) ☐ Below Poverty Line (2) ☐

15. **Habitat:** Urban (1) ☐ Semi-Urban (2) ☐ Rural (3) ☐

16. **Religion:** Hindu (1) ☐ Muslim (2) ☐ Sikh (3) ☐

Christian (4 ☐ Others (5)

17. **History of Present illness :** ----------

---

18. **History of Previous illness (If any):** ----------

Any significant medical / surgical history Yes (1) ☐ No (0) ☐

If Yes Specify ----------

CLINICAL PROFILE

19. **Personal History**

(i) Dietary Habits: Vegetarían (1) ☐ Non-Vegetarían (2) ☐

(ii) Addictions: Smoking (1) ☐ Tobacco(2) ☐ Alcohol(3) ☐ Drugs(4) ☐ None(5) ☐

(iii) Sleep: Normal (1) ☐ Abnormal(2) ☐

If abnormal, specify _____

(iv) Bowel Habits: Regular (1) ☐ Abnormal (2) ☐

If abnormal, specify _____

(v) Urine: Normal (1) ☐ Abnormal(2) ☐

If abnormal, specify _____

(vi) Allergy to some material: Yes (1) ☐ No (0) ☐

If yes, specify _____

(vii) Any emotional stresses:-Average(1) ☐ Moderate (2) ☐ Too Much (3) ☐

20. **EXAMINATION OF THE EYE**

Vision examination

Normal (1) ☐ Abnormal (2) ☐

If abnormal, specify_____

Movement

Normal (1) ☐ Abnormal (2) ☐

If abnormal, specify_____

Lacrimal System

Normal (1) ☐ Abnormal (2) ☐

(ashruyantra)

If 'abnormal' specify, _____

Conjunctiva (bulbar)

Congestion (GRADE 0-5) _____
Oedema (GRADE 0-5) _____
Haemorrhage (GRADE 0-5) _____
Redness (GRADE 0-5) _____
Nodule (GRADE 0-5) _____

Conjunctiva (tarsal)

Tarsal scarring (GRADE 0-5) _____
Fllicles (GRADE 0-5) _____
Others (GRADE 0-5) _____

Sclera (Sukla mandala)

Change in colour (GRADE 0-5) _____
Pigmentation (GRADE 0-5) _____
Nodule (GRADE 0-5) _____
Congestion (GRADE 0-5) _____

Cornea (Krishna mandala)

Lusture	Normal	☐	Lusterless	☐	
Vascularisation					
Sensation	Present	☐	Absent	☐	Reduced ☐
Epithelial status					
Tear film meniscus	Defect / erosion /desquamated				

21. **PHYSICAL EXAMINATION :**

Normal Abnormal

If abnormal', specify abnormalities _____

22. **SYSTEMIC EXAMINATION :**

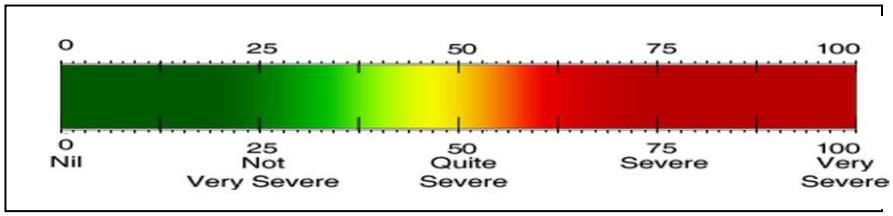
Normal Abnormal

If 'abnormal', specify abnormalities _____

23. **Assessment Parameters**

(A) Clinical parameters

Visual Analogue Scale



Parameters -	**Visual analogue Scale Score**
1. Feeling of dryness in the eyes,	☐
2. Burning sensation,	☐
3. Foreign body sensation (Sandy/ Scratchy / itching),	☐
4. Pricking pain,	☐
5. Rough lids / mucoid discharge/ mild blepharitis,	☐
6. Stuck eyelids,	☐

7. Blurred vision, ☐

8. Redness ☐

9. Narrowing of palpebral aperture ☐

(B) EYE TESTS

Tear film break-up test _____

Rose Bengal staining _____

Shchimer tests _____

Issue of trial drugs for 2 weeks

DY Eye drops Batch No. Quantity_____

Artificial tears Batch No. Quantity_____

Date to come on for next assessment_____

Name of the Investigator **Signature** **Date**

CLINICAL EVALUATION OF DARUHARIDRA (BERBERIS ARISTATA) - YASTIMADHU (GLYEYRRHIZA GLABRA) EYE DROPS IN THE MANAGEMENTOF DRY EYE SYNDROME (SHUSHKAKSHIPAKA)

CASE REPORT FORM III

(Enter a √ in the appropriate box)

Assessment on the 14th day

1. Code No (of clinical trial). _____

2. Centre : Ayurveda Central Research Institute (Ay.), New Delhi

3. Participant's Enrollment ID:_____

4. O.P. D. No: _____

5. Name of the Participant: _____

6) Assessment Parameters

A. Clinical parameters

Parameters	**Visual analogue Scale Score**
1. Feeling of dryness in the eyes,	
2. Burning sensation,	
3. Foreign body sensation (Sandy/ Scratchy / itching),	
4. Pricking pain,	
5. Rough lids / mucoid discharge/ mild blepharitis,	
6. Stuck eyelids,	
7. Blurred vision,	
8. Inflamation/ Redness	
9. Narrowing of palpebral aperture	

B. EyeTests

1. Tear film break-up test _____
2. Rose Bengal staining _____
3. Shchimer tests _____

Issue of trial drugs for 2 weeks

DY Eye drops Batch No. Quantity_____

Artificial tears Batch No. Quantity_____

Date to come on for next assessment_____

Name of the Investigator **Signature** **Date**

CLINICAL EVALUATION OF DARUHARIDRA (BERBERIS ARISTATA) - YASTIMADHU (GLYEYRRHIZA GLABRA) EYE DROPS IN THE MANAGEMENT OF DRY EYE SYNDROME (SHUSHKAKSHIPAKA)

CASE REPORT FORM III

(Enter a √ in the appropriate box)

Assessment on the 28th day

1. Code No (of clinical trial). _____
2. Centre : Ayurveda Central Research Institute (Ay.), New Delhi
3. Participant's Enrollment ID:_____
4. O.P. D. No: _____
5. Name of the Participant: _____

6. Assessment Parameters

A. Clinical parameters

Parameters	**Visual analogue Scale Score**
1. Feeling of dryness in the eyes,	
2. Burning sensation,	
3. Foreign body sensation (Sandy/ Scratchy / itching),	
4. Pricking pain,	
5. Rough lids / mucoid discharge/ mild blepharitis,	
6. Stuck eyelids,	
7. Blurred vision,	
8. Inflamation/ Redness	
9. Narrowing of palpebral aperture	

B. EyeTests

1. Tear film break-up test _____

2. Rose Bengal staining _____

3. Shchimer tests _____

Status of the patient:

Continuing (1) ☐ Drop out (2) ☐

Reason for drop out_____

Date: _____ Signature of Doctor _____

CLINICAL EVALUATION OF *DARUHARIDRA* (*Berberis aristata*) -*YASTIMADHU* (*Glyeyrrhiza glabra*) EYE DROPS IN THE MANAGEMENTOF DRY EYE SYNDROME (*Shushkakshipaka*)

PATIENT INFORMATION SHEET

1. Study title: Clinical Evaluationof *Daruharidra* (*Berberis aristata*) –*Yastimadhu* (*Glyeyrrhiza glabra*) Eye Drops in the management of Dry Eye Syndrome (*Shushkakshipaka*)

2. Invitation

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve, please take time to read the following information carefully and discuss it with friends and relatives if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

3. What is the purpose of the study?

To evaluate clinical efficacy of *Daruharidra* (*Berberis aristata*)–*Yastimadhu* (*Glyeyrrhiza glabra*) Eye Drops in the management of Dry Eye Syndrome (*Shushkakshipaka*)

4. Why have I been chosen?

Being a patient of Dry Eye Syndrome you are considered as an ideal candidate for the study.

5. Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be giventhis information sheet to keep and be asked to sign a consent form later. If you agree to take part you are still free to withdraw at any time and without giving any reason. This will not affect the standard of care you receive.

6. What will happen to me if I take part?

If you agree to take part in this study you will be prescribed Ayurvedic formulations which will be given for 4 weeks if you give your consent to continue in the study. You have to come at every 2weeks for follow-up clinical examination and for collecting the medicine for the next 2 weeks. You have to undergo general physical examination and laboratory investigations from time to time for the assessment of the effect of the medicine you would be taking at that time. Thereafter, in the subsequent visits it may take nearly 15 – 20 minutes to make the assessment.

7. What do I have to do?

You have to adhere to the instructions given to you by your Investigator regarding taking the medicines as advised and reporting for follow up on the prescribed day.

9. What are the alternatives for treatment?

The modern system of treatment of Dry Eye Syndrome involves the instillation of lubricating eye drops (Artificial Tears) in the eyes several times a day. The present study is being undertaken to scientifically study and validate the effect of this Ayurvedic Eye drops.

12. What if new information becomes available?

If during the course of the clinical trial some new information becomes available about theAyurvedic treatment being studied, you will be informed about that by your investigator after which you are free to decide whether you want to continue in the study or not. If you decide to withdraw, this will not adversely affect your routine care in the hospital. If you decide to continue in the study, you will be asked to sign a fresh consent form. On the other hand upon receiving new information your investigator might consider it to be in your best interests to withdraw you from the study. Your investigator will explain the reasons for dropping you from the study and arrange for your routine care to continue.

13. What will happen to the results of the research study?

The results of the clinical trial will be published in leading medical journals so that other doctors and researchers can benefit from the results. You can ask your investigator for a copy of the publication. If published, your identity and personal details will be kept strictly confidential. No named information about you will be published in any of the trial reports.

14. Contact for further information

If desirous of any relevant information at any stage of the clinical trial, you may feel free to ask your investigator for that information. You would be given a copy of the information sheet and a signed consent form.

CLINICAL EVALUATION OF *DARUHARIDRA* (*Berberis aristata*) -*YASTIMADHU* (*Glyeyrrhiza glabra*) EYE DROPS IN THE MANAGEMENT OF DRY EYE SYNDROME (*Shushkakshipaka*)

CONSENT FORM

TO BE SIGNED ON THE DAY OF SCREENING

1. Centre ----------
2. Participant enrollment ID for this trial: ----------
3. Name of the Investigator ----------
4. I confirm that I have read / the study has been explained to me adequately and I have understood the information sheet for the above study and had the opportunity to ask questions.
5. I hope to complete the study, but I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason, and without my medical care or legal rights being affected.
6. I understand that my doctor will provide information about my progress,
7. I understand that the information will be used for medical research only and that I will not be identified in any way in the analysis and reporting of the results. I understand that sections of any of my medical notes may be looked at by the Sponsors or responsible individuals from the members of the IEC, Regulatory authorities. Ifnecessary. I give permission for these individuals to have access to my records.
8. I understand what is involved in this trial and agree to take part in the clinical trial for a period of4 weeks.

Name of the Patient	Signature	Date
Name of the witness	Signature	Date
Name of the Investigator	Signature	Date

Annexure-2

Published Research Papers

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DEVELOPMENT AND STANDARDIZATION OF AN AYURVEDIC HERBAL EYE DROPS FOR DRY EYE SYNDROME

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ABSTRACT

Ayurvedic literatures recount potential ophthalmicdrugs for the management of surface inflammatory conditions of eye such as dry eye syndrome(DES) or Keratoconjunctivitis Sic ca(KCS). The plant drugs such as *Berberi saristata* DC. and *Glyeyrrhiza glabra* Linn. are attributed with potential anti-inflammatory, anti-allergic and wound healing activities backed by scientific evidences. With this rationale and background, an Ayurvedic eye drops was developed for dry eye syndrome (DES) systematically following contemporary methods and parameters right from quality assurance of ingredients, formulation of standard operation procedures (SoPs) and also complying to the quality and safety standards of finished product for ophthalmic preparation as specified in Indian Pharmacopeia. The pre-clinical ocular toxicity studies also revealed safety of this formulation on topical use.

KEYWORDS: eye drops, dry eye syndrome, Standardization, TLC.

INTRODUCTION

Dry eye syndrome (DES) is an eye disease, which, in turn, is caused by either decreased tear production or increased tear film evaporation.[1] The Latin phrase "kerato-conjunctivitissicca" indicates dryness and inflammation of the cornea and conjunctiva. Ayurvedic literatures describe DESas*sshushkakshipaka, parishuskha-netra,ativishuskha--netra, asrusravarahita-netra*and*asnigdha-netra*indicative of dryness of eye due to deficiency in tear film components.[2] There are many conditions which cause dryness of the eyes such as hypo function of lacrimal glands, mucin deficiency, conjunctival scarring etc.[3] Dry eye syndrome

is the most common eye disease, affecting 5 - 6% of the population.[4,5,6] Management strategies of dry eye syndrome include mainly supplementation of tear preferably substitutes containing methylcellulose or carboxymethylcellulose or identical substances which are viscous in nature.[7] The tear stimulants such as cholinergic drugs increases the tear production from lacrimal gland by stimulating secretions, but not been used in clinical practice. All these drugs do not have any effect on basic path physiology and they provide only symptomatic relief.[8]

BASIS FOR FORMULATION HERBAL EYE DROPS

Owing to limitations of different conventional agentsit is at this juncture that the need for safe and effective drugs that could effectively tackle dry eye syndrome. A vast number of indigenous drugs coupled with innumerable claims of their varied uses in alleviating wide range of ophthalmic affections calls for scientific validation for their safety and efficacy. Ayurvedic literatures record more than fifty ophthalmic plant drugs and more than forty metals minerals having diversified pharmacological actions on visual system and adnexa of the eye.[9,10,11]

Yastimadhu(*Glyeyrrhiza glabra* Linn.) & Daruharidra(*Berberis aristata* DC.) are some of such medicinal plant sources having potential leads in the management of surface ocular inflammatory conditions such as dry eye syndrome, supported by textual references from Ayurvedic literatures backed by experimental and clinical studies. A study intervention comprising of topical and internal use of *Daruharidra*(*Berberis aristata*) has shown significant improvement in subjective parameters like dryness, redness, photophobia etc. in dry eye syndrome.[12,13,14] Pharmacological actions such as *caksusya* (conducive to vision), *netrya* (conducive to adnexa of eye), *netraruja-hara* (analgesic ophthalmic action), netra-*sodhahara* (anti-inflammatory action) *netrakanduhara* (anti allergic action), *vrana-ropana*(wound healing effect) are attributed to these drugs.[9,15,16] Yastimadhu(*Glyeyrrhiza glabra*)has shown notable anti-inflammatory action attributed to cortisone-like substance present in this plant that helps reduction of inflammation.[17] It is evident that the combination of ingredients viz. Yastimadhu(*Glyeyrrhiza glabra*), Daruharidra (*Berberis aristata*) certainly play a significant role in restoring the functions of tear film, prevention of ulceration and related checking inflammatory process and contributory to the comprehensive management of dry eye syndrome. The eye drops is formulated with these two plant ingredients and developed as per Indian Pharmacopeia (IP, 1996) complying quality standards and other

parameters such as isotonic to lacrimal fluid, particulate matter, pH, Sodium chloride content, sterility test besides permissible preservatives and packing specifications etc.[18,19,20]

METHODOLOGY AND OBSERVATIONS

Raw drug identification and quality assurance: Raw ingredients viz. dried unpeeled stolon and root of *Yastimadhu* (*Glycyrrhiza glabrla*Linn.) and dried stem of *Daruharidra* (*Berberis aristata* DC.) procured from authentic market sources**(fig-1).** The identity was confirmed with compliance of microscopic, macroscopic parameters of Ayurvedic pharmacopoeia of India (API) hrough pharmacognosy studies.[22,25] The purity and strength were also confirmed through physico-chemical studies done as per 'Protocol For Testing of ASU Drugs (2008')', Pharmacopeial Laboratory for Indian Medicine, Ministryof AYUSH, Govt. India[23] and compliant with parameters of Ayurvedic pharmacopoeia of India(API).[22,25] **(table-1 and table-2).**

Fig.1-Samples of ingredients of Herbal Eye Drops

***Daruharidra*(*Berberis aristata*DC.)** | ***Yastimadhu* (*Glycyrrhiza glabrla*Linn.)**

Table-1.Physico-chemical Studies of *Yastimadhu* (*Glycyrrhiza glabrla*Linn.)

SNo.	Parameters	Results
1.	Total ash	7.92
2.	Acid –insoluble ash	0.62
3.	Water soluble extractive	25.69
4.	Alcohol soluble extractive	23.37
5.	pH (1% w/v aqueous solution)	5.52

Table-2.Physico-chemical Studies of *Daruharidra*(*Berberis aristata*DC.)

SNo.	Parameters	Results
1.	Loss on drying at 105°C (% w/w)	5.13
2.	Total ash	2.10
3.	Acid-insoluble ash (% w/w)	0.01
4.	Water-soluble extractive (% w/w)	10.64
5.	Alcohol soluble extractive (% w/w)	6.45

Standard Operative Procedures (SoPs) for eye drops development and analytical specifications: The step wise development of eye drops encompass the preparation of distillate, making of the distillate isotonic to lacrimal fluid and adjustment of pH, addition of preservative and packing under sterile conditions. 50g powder (particles passed through 40-mesh) of each of dried unpeeled stolon and root of *Yastimadhu* (*Glycyrrhiza glabrla* Linn.) and dried stem of *Daruharidra* (*Berberis aristata*DC.) of pharmacopeia quality was soaked in 850 ml of distilled water for overnight in an air tight container. The material was transferred to a distillation unit. Distillate was obtained by adjusting the temperature to 40^{o}C for 15 minutes and raising the temperature slowly to 80^{o} C. The first 450 ml. of distillate was collected at the rate of 20 drops per minutes in anairtight container. The distillate was made isotonic to lacrimal fluid by adding 0.9% NaCl to distillate and dissolving properly and adding isotonic phosphate buffer viz. 0.16 g. of monobasic Sodium phosphate and 0.76g of dibasic Sodium phosphate. Finally the pH of the eye drops was adjusted to 6.9-7.30. Benzalkonium chloride in a ratio of 1:10000, was added as preservative and pH was again checked and found within the specified range of the ophthalmic drops (pH 6.9-7.30). Test for sterility performed after addition of preservative the preparation was observed for 48hours, and found sterile. The packing was made in autoclaved sterilized amber glass containers of 10 ml. Capacity**.**The finished product tested for quality assurance and safety and the analytical specifications complied specified parameters of Indian pharmacopeia for ophthalmic preparations (**table-3).**

Table-3. Analytical Specifications of Herbal eye drops

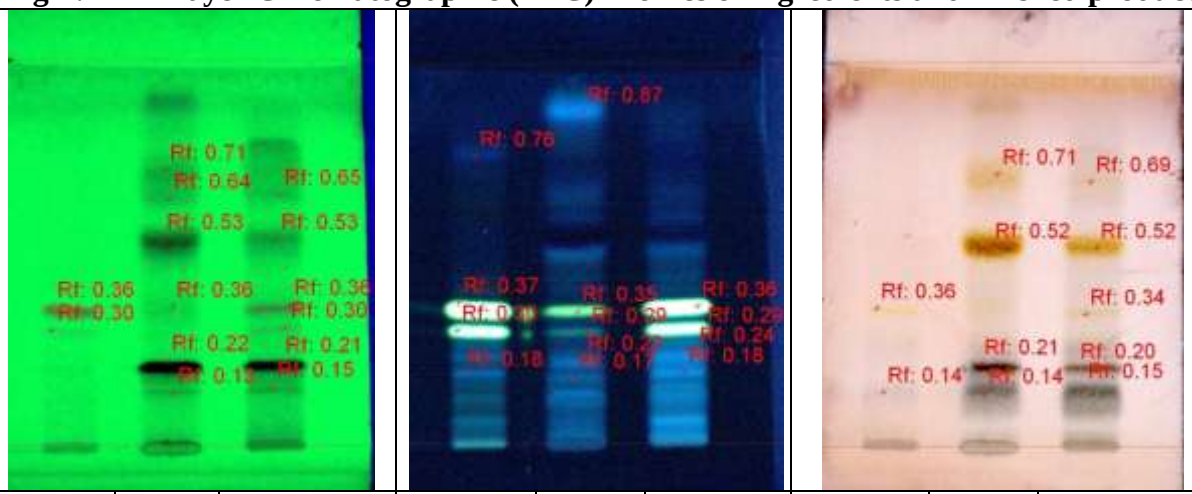
Descriptions	:	Colorless clear solution with mild characteristic odor
Particulate matter	:	Passes as per Indian Pharmacopeia, 1996. (when examined under suitable conditions of visibility, are clear and practically free from particles that can be observed on visual inspection by unaided eye.)
Identification	:	Passes *Liberman Burchard* test Extract 10 ml. eye drops in vol. of extract to one ml. add one drop of acetic anhydride and keep it for a drop of conc. Sulphuric acid from the side of test tube a violet color ring appears at the junction of two liquids which gradually disappears.
pH	:	6.9-7.3
Sodium chloride content(%)	:	0.85-0.95% w/v
Sterility test	:	Passes (Indian Pharmacopeia,1996)

Quality standards of herbal eye drops: The thin layer chromatographic (TLC) profiling of raw ingredients of *Yastimadhu* (*Glycyrrhiza glabrla* Linn.) *Daruharidra* (*Berberis aristata* DC.) was done in the following way. 1.0g each of *these* ingredients were soaked overnight separately in 10 ml of 70% methanol. The solutions were continuously stirred for 6 hrs and kept for next 18 hrs. Next day filtered the samples, dried and Made 10% solutions. Now 50ml of sample (eye drop), was concentrated to volume of 1.0ml over waterbath maintained at 80-90^{o}C & add 1.0ml of methanol. 5µl each of these solutions were applied separately in 10mm band length on Merck Aluminum plate pre-coated with silica gel 60 F_{254} of 0.2mm thickness with the help of Linomat IV applicator. The plate was developed in Twin trough glass chamber using mobile phase n-Butanol: Water: Acetic Acid (7:2:1). The plate was dried in air and visualized under λ 254 nm and λ 366 nm for ultra violet detection and taken the fingerprints. Later the plate was dipped in Anisaldehyde – Sulphuric acid and heated at 105°C till the colour of the spots appeared and fingerprint taken under white light. The TLC profiling of finished product of eye drops was matched with that of ingredients (**table-4) & (fig-2).**

Table-4 Observations of Thin Layer Chromatographic (TLC) Profiles of ingredients and finished product

Sample	Visualization/ Detection (R_f Values)		
	Under UV 254nm	Under UV 366nm	Derivatized
Berberis aristata DC.	0.30, 0.36	0.18, 0.30, 0.37, 0.76	0.14, 0.36
Glycyrrhiz aglabra Linn.	0.13, 0.22, 0.36, 0.53, 0.64, 0.71	0.17, 0.22, 0.29, 0.35, 0.87	0.14, 0.21, 0.52, 0.71
Eye drop(finished product)	0.15, 0.21, 0.30, 0.36, 0.53, 0.65	0.18, 0.24, 0.29, 0.36	0.15, 0.20, 0.34, 0.52, 0.69

Fig-2. Thin Layer Chromatographic (TLC) Profiles of ingredients and finished product



A	B	C	A	B	C	A	B	C
254nm			366nm			Derivatized		

A-*Berberis aristata* DC., *B-Glycyrrhiza glabra* Linn., C- Eye drop (*formulation)*

Preclinical Safety studies: Acute Eye Irritation tests revealed that the eye drops did not cause irritation to ocular mucous membrane of eyes of rabbits such as opacity ulceration of cornea; congestion, swelling, moderate circum-corneal hyperaemia; injection, hemorrhage, gross destruction of iris; redness and other signs of inflammation of palpebral and bulbar conjunctivae; chemosis of lids etc.and no evident signs of toxicity were observed. Further, no clinical signs of mortality and change in body weight were noticed during the observation period of 72 hours of post application, was found to be practically nonirritant to the eyes of rabbits.[24]

CONCLUSION

In spite of great technological advances in the field of ophthalmic medicine and surgery, conservative therapy still continues to be mainstay for reversible ailments. Researchers are relentlessly in quest to identify plants metals and minerals with medicinal properties. Often they are successful, proverbially, in turning over a new leaf. At the same time, there are numerous challenging problems, existing before modern ophthalmologists that require special attention to develop unexplored fields of medical knowledge hidden in ancient medical texts. Contemporary management strategies of dry eye syndrome though clinically effective poses certain challenges and limitations. Considering this, it is pivotal to translate some potential leads for management of dry eye syndrome as detailed in Ayurvedic texts into user friendly-safe, effective and quality assured dosage forms to improve patient's compliance. The herbal eye drops developed certainly contribute significantly in the management of dry eye syndrome.

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OCULAR SAFETY AND TOXICITY STUDIES OF AN AYURVEDIC HERBAL EYE DROP FOR DRY EYE SYNDROME

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ABSTRACT

Acute Eye Irritation study of an Ayurvedic herbal eye drop containing herbal ingredients viz. *Berberis aristata* and *Glyeyrrhiza glabra* formulated for dry eye syndrome (DES) complying the standards of Indian Pharmacopeia (IP.) was performed in New Zealand White rabbit as per the OECD guidelines for testing of chemicals (acute eye Irritation/corrosion) after fulfilling ethical requirements. 0.1 ml of the drop was placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The other eye, which remains untreated, serves as a control. Eye drop did not cause irritation to mucous membrane of eyes of rabbits, no evident signs of toxicity and clinical changes were observed. The eye drop in New Zealand White rabbits was found to be nonirritant to the ocular mucous membrane.

KEY WORDS *Berberis aristata*, *Glyeyrrhiza glabra*, Acute Eye Irritation study,

INTRODUCTION

Kerato-conjunctivitis sicca (KCS), or dry eye syndrome (DES) is an eye disease caused by eye dryness, which, in turn, is caused by either decreased tear production or increased tear film evaporation.[1] The Latin phrase "kerato-conjunctivitis sicca" indicates dryness and inflammation of the cornea and conjunctiva. Ayurvedic literatures describe DES as *shushkakshipaka, parishuskha-netra, ativishuskha--netra, asrusravarahita-netra* and *asnigdha-netra* indicative of dryness of eye due to deficiency in tear film components.[2] There are many conditions which cause dryness of the eyes such as hypo function of lacrimal glands, mucin deficiency, conjunctival scarring etc.[3] Dry eye syndrome is the most common eye disease, affecting 5 - 6% of the population.[4,5,6] Managementstrategies of DES include mainly supplementation of tear preferably substitutes containing methylcellulose or carboxymethylcellulose or identical substances which are viscous in nature.[7] The tear stimulants such as cholinergic drugs increases the tear production from lacrimal gland by stimulating secretions, but not been used in clinical practice. All these drugs do not have any effect on basic path physiology and they provide only symptomatic relief.[8]

Owing to challenges of different conventional agents, it is at this juncture that the need for safe and effective drugs that could effectively tackle DES .A vast number of indigenous drugs coupled with innumerable claims of their varied uses in alleviating wide range of ophthalmic affections calls for scientific validation for their safety and efficacy. Ayurvedic literatures record more than fifty ophthalmic plant drugs and more than forty metals minerals having diversified pharmacological actions on visual system and adnexa of the eye.[9,10,11]

Adding to the references from Ayurvedic texts, few clinical studies on medicinal plants such as Yastimadhu (*Glyeyrrhiza glabra*), Daruharidra (*Berberis aristata*) also endorse the usefulness of these plants in surface inflammatory ocular lesions such as dry eye syndrome. A study intervention comprising of topical and internal use of *Daruharidra* (*Berberis aristata*) has shown significant improvement in subjective parameters like dryness, redness, photophobia etc. in DES.[12,13,14] Pharmacological actions such as *caksusya* (conducive to vision), *netrya* (conducive to adnexa of eye), *netraruja-hara* (analgesic ophthalmic action), netra-*sodhahara* (anti-inflammatory action) *netrakanduhara* (anti allergic action), *vrana-ropana* (wound healing effect) are attributed to these drugs.[9,15,16] Yastimadhu (*Glyeyrrhiza glabra*) has shown notable anti-inflammatory actionattributed to cortisone-like substance present in this plant that helps reduction of inflammation.[17] It is evident that the combination

of ingredients viz. Yastimadhu (*Glyeyrrhiza glabra*), Daruharidra (*Berberis aristata*) certainly play a significant role in restoring the functions of tear film, prevention of ulceration and related checking inflammatory process and contributory to the comprehensive management of DES. The Ayurvedic herbal eye drops was formulated with these two plant ingredients and developed as per Indian Pharmacopeia (IP.1996) complying quality standards and other parameters such as isotonic to lacrimal fluid, particulate matter, pH, sodium chloride content %, sterility test besides permissible preservatives and packing specifications etc.[18,19,20]

MATERIALS AND METHODS

Objective: The objective of this Acute eye irritation study in rabbit was to assess the toxic characteristics of the Ayurvedic herbal eye drop when instilled in to rabbit eye in a single dose. The study conducted in full compliance with the guidelines laid down in OECD 405 - OECD Guideline for the testing of chemicals (Acute Eye Irritation/Corrosion) Adopted 24 April 2002 and other prevalent guidelines.[21, 22, 23]

Test System		
Test system	:	Rabbit
Strain	:	New Zealand White
Age	:	12 to 14 weeks
Body weight range	:	1.925 kg to 2.180 kg
Identification	:	By cage tag and corresponding colour body markings
Number & sex of animals	:	Six (3 males +3 females)
Acclimatization	:	One week in experimental room after veterinary examination
Randomization	:	After acclimatization and veterinary examination animals were randomly selected.
Nutritional conditions	:	Fasted four hours prior to treatment. Food was offered one hour after dosing.
Environmental conditions	:	Air conditioned rooms with 10 - 15 air changes per hour, temperature between 21±2 0C, relative humidity 55 ±5 % and illumination cycle set to 12 hours artificial fluorescent light and 12 hours dark.=
Accommodation	:	Individually housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
Diet	:	Nutrimix' brand pelleted standard rabbit feed Manufactured by Nutrivet Life Sciences, Pune, was provided *ad libitum*.
Water	:	Potable water passed through reverse osmosis filtration system and exposed to UV ray was provided ad libitum in glass bottles with stainless steel sipper tubes.

Preparation of Animals: Both eyes of each experimental animal provisionally selected for testing were examined within 24 hours before testing starts. Animals showing eye irritation, ocular defects, or pre-existing corneal injury will not be used.

Study Design: After an Acclimatization period and pre examination the rabbits were weighed and the required numbers of animals were randomly allocated to the treatment group. As described below, this group of rabbits was instilled eye drop (0.1 ml) into the right eye and was observed for the eye irritation and clinical sign for 72 hours. The untreated eye serves as the control.

Group No.	Dose (ml)	Female Rabbits	
		Numbers	ID
1	0.1	6	184/RB001 – 184/RB006

Administration: One day prior to treatment, the eyes of all the rabbits were examined. The eye drop (0.1 ml) was into the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The other eye, which remains untreated, serves as a control.

Observations

Mortality: On the day of administration, all animals were observed for mortality at 30 min, 1, 2, 4 and 6 hours following topical application and thereafter they were observed once a day for 72 hours.

Clinical Signs and Grading of Reactions: The treated animals were observed for signs of intoxication, at 10, 30 min, 1, 2, 4 and 6 hours after application and thereafter once a day for 72 hours. The appearance, progress and disappearance of the signs if any were recorded. Changes, if any, in gait, posture and responses to handling as well as the presence of clonic or tonic movements, stereotypies or bizarre behavior were also recorded.

The eyes were examined at 1, 24, 48, and 72 hours after test substance application. The grades of ocular reaction (conjunctivae, cornea and iris) were recorded at each examination. Examinations of reactions were facilitated by use of a binocular loupe, hand ophthalmoscope (Table 1).

Table 1: Grading of ocular lesions

A .Cornea	
Opacity: degree of density (readings should be taken from most dense area)*	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible	1
Easily discernible translucent area; details of iris slightly obscured	2
Nacrous area; no details of iris visible; size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Maximum possible: 4	
* The area of corneal opacity should be noted	
B. Iris	
Normal	0
Deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect	1
Hemorrhage, gross destruction, or no reaction to light	2
Maximum possible: 2	
C. Conjunctivae	
Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)	
Normal	0
Some blood vessels hyperaemic (injected)	1
Diffuse, crimson colour; individual vessels not easily discernible	2
Diffuse beefy	3
Maximum possible: 3	
D. Chemosis	
Swelling (refers to lids and/or nictating membranes)	
Normal	0
Some swelling above normal	1
Obvious swelling, with partial eversion	2
Swelling, with lids about half closed	3
Swelling, with lids more than half closed	4
Maximum possible: 4	

Body weights: The body weights of rabbits were individually recorded before application of eye drop.

RESULTS

Changes in Ocular mucous membrane: The Ayurvedic herbal eye drop did not cause irritation to mucous membrane of eyes of rabbits and no evident signs of toxicity were observed. The eye drop in New Zealand White rabbits was found to be nonirritant to the ocular mucous membrane. The data of the individual animal ophthalmoscopy report, individual score for occular irritation, irritation index and reference values for eye irritation are given in the Table 2 – Table 5.

Clinical Signs and Mortality: The Ayurvedic herbal eye drop tested (0.1ml), for Acute Eye Irritation test did not cause any mortality and no evident sings of toxicity observed, during the observation period of 72 hours post application.

TABLE 2: Individual animal ophthalmoscopy report

Dose: 0.1 ml/Rabbit Group: G1 Sex: M / F Study day: 0

ANIMAL ID	184/RB001	184/RB002	184/RB003	184/RB004	184/RB005	184/RB006
Area examined						
Retinal Reflex	N	N	N	N	N	N
Lids ADNEXA Ducts, Cornea	N	N	N	N	N	N
Iris	N	N	N	N	N	N
Aqueous Humour	N	N	N	N	N	N
Lens	N	N	N	N	N	N
Vitreous Humour	N	N	N	N	N	N
Retina [Vessels [Macula	N	N	N	N	N	N
Optic Disc	N	N	N	N	N	N
Tapetum Lucidum	N	N	N	N	N	N
Tapetum Nigrum	N	N	N	N	N	N

TABLE 3: Reference values for eye irritation[24]

MMTS	Irritation Classification	Requirement for maintenance of classification
0.0 – 0.5	Non	Up to 0.5 at 1 hour with zeros at 24 hours; otherwise, increase one level
0.6 – 2.5	Partially Non	With zeros at 24 hours; otherwise, increase one level
2.6 – 15.0	Minimally	With zeros at 48 hours; otherwise, increase one level
15.1 – 25.0	Mildly	With zeros at 96 hours; otherwise, increase one level
25.1 – 50.0	Moderately	With 7 day mean ≤ 20 and individual total scores ≤ 10 in at least 60% of the rabbits with no total score > 30; otherwise, increase one level
50.1 – 80.0	Severely	With 7 day mean ≤ 40 and individual total scores ≤ 30 in at least 60% of the rabbits with no total score > 60; otherwise, increase one level.
80.1 –100.0	Extremely	With 7 day mean ≤ 80 and individual total scores ≤ 60 in at least 60% of the rabbits with no total score > 100; otherwise, increase one level.
100.1 - 110	Maximally	With 7 day mean > 80 and individual total scores >60 in at least 60% of the rabbits; otherwise, decrease one level.

Table 4: Individual score for occular irritation

	Scoring (at hours)											
Animal ID	**184/RB001**				**184/RB002**				**184/RB003**			
	1	24	48	72	1	24	48	72	1	24	48	72
I Cornea												
A. Opacity	0	0	0	0	0	0	0	0	0	0	0	0
B. Area	0	0	0	0	0	0	0	0	0	0	0	0
(AX B) X 5	0	0	0	0	0	0	0	0	0	0	0	0
II Iris												
A. Values	0	0	0	0	0	0	0	0	0	0	0	0
A X 5	0	0	0	0	0	0	0	0	0	0	0	0
III Conjunctivae												
A. Redness	0	0	0	0	0	0	0	0	0	0	0	0
B. Chemosis	0	0	0	0	0	0	0	0	0	0	0	0
C. Discharge	0	0	0	0	0	0	0	0	0	0	0	0
(A+B+C) X 2	0	0	0	0	0	0	0	0	0	0	0	0
Total score	0	0	0	0	0	0	0	0	0	0	0	0
Average score	0	0	0	0	0	0	0	0	0	0	0	0
MMTS	0	0	0	0	0	0	0	0	0	0	0	0

MMTS= Maximum Mean Total score

Cornea (AXB) X 5= Total max. 80, Iris AX5 = Total max. 10, Conjunctivae (A+B+C) X 2= Total max. 20

Table 5: Irritation Index

Total Number of Rabbits		**Hours**			
		1	**24**	**48**	**72**
6	**MMTS**	0	0	0	0
	Maximum Average Irritation Index	0	0	0	0

The incidence, severity and reversibility of irritation data

Time post Instillation	**Incidence of Irritation**		
	Corneal opacity	**Iritis**	**Conjunctivitis**
1 Hour	0/6	0/6	0/6
24 Hours	0/6	0/6	0/6
48 Hours	0/6	0/6	0/6
72 Hours	0/6	0/6	0/6

Time post Instillation	**Severity of Irritation Mean score**
1 Hour	0
24 Hours	0
48 Hours	0
72 Hours	0

CONCLUSION

The Ayurvedic herbal eye drop did not cause irritation to ocular mucous membrane of eyes of rabbits such as opacity ulceration of cornea; congestion, swelling, moderate circumcorneal hyperaemia; or injection, hemorrhage, gross destruction of iris; redness and other signs of inflammation of palpebral and bulbar conjunctivae; chemosis of lids etc. and no evident signs of toxicity were observed. Further, no clinical signs of mortality and change in body weight were noticed during the observation period of 72 hours post application and the Ayurvedic herbal eye drop was found to be practically nonirritant to the eyes of rabbits.

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IN VITRO BIOCHEMICAL ASSESSMENT OF ANTIOXIDANT POTENTIAL OF AN AYURVEDIC HERBAL EYE DROPS FOR DRY EYE SYNDROME

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ABSTRACT

Dry Eye Syndrome is a leading cause of ocular discomfort affecting millions of people. Dry Eye conditions are a spectrum of disorders with varied aetiology ranging from mild eyestrain to very severe dry eyes associated with visual complications. Dry eye is a condition produced by the inadequate interrelation between lachrymal film and ocular surface epithelium, and is caused by quantitative and qualitative deficits in one or both of them. Ocular surface conditions may result from the abnormalities in one or more of the tear film components, ocular or systemic diseases, various drugs and even environment factors. Oxidative stress is involved in many surface ocular diseases including dry eye syndrome (DES). The anterior eye segment and mainly the cornea are directly affected by the hazards from potential oxidative damage evoked by air pollution, radiation, chemicals as evident by proven role of oxidative stress in the pathogenesis surface ocular disease. Further certain scientific studies have demonstrated beneficial effects and protective role of topical and oral antioxidants in the management of surface lesions like dry eye disease. Along with measures such as tear preservation, use of tear stimulants, tear substitutes and control of infection, the advocacy of topical and oral antioxidant agents also forms an important component in the management of dry eye syndrome (DES). Owing to its importance, the biochemical assessment of antioxidant potential of the Ayurvedic herbal eye drops formulated for dry eye syndrome was performed. The plant based ingredients of the eye drop possess promising leads for their antioxidant, anti-inflammatory; anti-microbial potential. The study encompasses biochemical antioxidant assays viz. Nitric oxide radical scavenging assay, ABTS radical scavenging assay, DPPH radical scavenging assay and Ferric reducing antioxidant potential (FRAP) assay. These studies revealed significant antioxidant potential of herbal eye drops, possibly compliment to the management of dry eye syndrome and marinating the surface ocular health.

KEYWORDS: antioxidant activity, Ayurvedic herbal eye drop, dry eye syndrome.

INTRODUCTION

Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tears film instability with potential damage to the ocular surface. It is accompanied by increased osmolality of the tear film and inflammation of the ocular surface. Based on the pathophysiology of tear film formation the classification of DES suggested by Holly and Lemp comprise Aqueous Tear Deficiency (ATD); Lacrimal surfactant (mucin) deficiency; Lipid layer abnormality; Impaired lid function or blinking; and Epitheliopathy.[1,2] The Latin phrase "kerato-conjunctivitissicca" indicates dryness and inflammation of the cornea and conjunctiva. Ayurvedic literatures describe DES as *shushkakshipaka, parishuskha-netra, ativishuskha--netra, asrusravarahita-netra* and *asnigdha-netra* indicative of dryness of eye due to deficiency in tear film components.[3] There are many conditions which cause dryness of the eyes such as hypo function of lacrimal glands, mucin deficiency, conjunctival scarring etc.[4] Dry eye syndrome is the most common eye disease, affecting 5 - 6% of the population.[5,6,7] Further it became a significant public health problem distributed among 10% of the adult population and 18% of the elderly population.[8]

Contemporary management strategies for Dry eye syndrome pose certain limitations. Management strategies of DES include mainly supplementation of tear preferably substitutes containing methylcellulose or carboxy-methylcellulose or identical substances which are viscous in nature**.** Preservatives used in formulations are known to cause dry eyes. All these drugs do not have any effect on basic path physiology and they provide only symptomatic relief.[9] The tear stimulants such as cholinergic drugs increases the tear production from lacrimal gland by stimulating secretions, but not been used in clinical practice. Tear Preservation can be done by occluding the puncta or minimizing evaporation, But is useful as short-term measure to assess the effect of occluding puncta before resorting to permanent measures. All these drugs do not have any effect on basic path physiology and they provide only symptomatic relief.[10] Further Topical antibiotics and corticosteroids are sometimes used to treat secondary infections and inflammation. But discontinuation of antibiotics, steroids and all preservative-containing eye drops is mandatory for relief of symptoms and progressively improving the tear film and ocular surface. In view of the above, there is an urgent need to evolve safe and effective management approach to tackle symptoms, infections and underlying pathology.

Protective role of antioxidant supplementation in the maintenance ocular surface health: Oxidative stress in the cornea influenced by several environmental factors such as air pollution, radiation, chemicals etc. leads to changes in corneal optical properties and decrease in visual acuity or even vision loss. The antioxidant agents help in suppressing the damages due to oxidative stress hand assist in restoring the corneal health.**[11]** The surface lesions and corneal diseases, associated with oxidative stress leads to corneal aging, b corneal inflammation In acute corneal inflammation the Reactive oxygen species (ROS) are highly involved. Studies on the oxidative reactions in tears of patients with dry eye disease confirmed a marked increase of inflammatory activity in the tear film of patients suffering from dry eye. These reactions lead to severe damage of the eye. Free radicals and inflammation may be involved in the pathogenesis or in the self-propagation of the dry eye disease**.** The antioxidant therapy with superoxide dismutase and dimethylthiourea are employed for the healing of corneal ulcers evoked by sodium hydroxide. Topical antioxidant therapy found effective in reducing the inflammatory corneal reaction.[12-18]

Further, the Antioxidant supplements such as vitamin C and vitamin E, probably have an important role in reducing the oxidative damage produced by nitric oxide and other free radicals and improving the ocular surface milieu.[19]

Need for development of Inclusive therapeutic strategies: in view of challenges of different conventional agents to manage dry eye disease. It is imperative to develop such topical dosage form which could offer safe, effective and comprehensive management. Ayurvedic literatures record more than fifty ophthalmic plant drugs and about forty metals minerals having diversified pharmacological actions on visual system and adnexa of the eye.[20,21,22] Adding to the references from Ayurvedic texts,few clinical studies on medicinal plants such as *Yastimadhu* (*Glyeyrrhiza glabra*) and *Daruharidra* (*Berberis aristata*) also substantiate the usefulness of these plants in surface inflammatory ocular lesions such as dry eye syndrome. A study intervention comprising of topical and internal use of *Daruharidra* (*Berberis aristata*) has shown significant improvement in subjective parameters like dryness, redness, photophobia etc. in DES. [23,24,25] Pharmacological actions such as *caksusya* (conducive to vision), *netrya* (conducive to adnexa of eye), *netraruja-hara* (analgesic ophthalmic action), netra-*sodhahara* (anti-inflammatory action) *netrakanduhara* (anti allergic action), *vrana-ropana* (wound healing effect),anti-bacterial actions are attributed to these drugs.[26,27] *Yastimadhu* (*Glyeyrrhiza glabra*) has shown notable anti-inflammatory action attributed to cortisone-like substance present in this plant that helps reduction of inflammation.[28] It is evident that the combination of ingredients viz. *Yastimadhu* (*Glyeyrrhiza glabra*), Daruharidra (*Berberis aristata*) may play a significant role in restoring the functions of tear film ,prevention of ulceration and related checking inflammatory process, infection and contributory to the comprehensive management of DES.

The medicinal plant ingredients rationally chosen in formulating the eye drops for dry eye disease viz. *Glycyrrhiza glabra* and *Berberis aristita* possess antioxidant, antimicrobial activity backed by scientific evidence. Methanolic extract of *Berberis aristita* has shown DPPH free radical Scavenging Activity expressed in % inhibition with L Ascorbic acid as standard showing IC50 9.6µg/ml and that of extract was 33.31µg/ml. Hydrogen peroxide radical scavenging activity was comparable to standard IC50 for L Ascorbic acid is 54.23µg/ml and that of *B. aristita* is 60.6µg/ml. Similarly, reducing power of plant extract at different concentration was comparable with L-Ascorbic Acid. The Antimicrobial screening revealed activity against *Candida albicans, Salmonella typhii, Pseudomonas aeruginosa and Escherichia coli* [29] In an experimental study, the antioxidant assay of methanaloic extract of *Glycyrrhiza glabra* confirmed the potent antioxidant activity.[30]

The eye drop is formulated with these two plant ingredients and developed complying quality standards and other safety parameters.[31,32,33] Ocular toxicity and safety studies revealed The eye drops did not cause irritation to ocular mucous membrane of eyes of rabbits such as opacity ulceration of cornea; congestion, swelling, moderate circumcorneal hyperemia; or injection, haemorrhage, gross destruction of iris; redness

and other signs of inflammation of palpebral and bulbar conjunctivae; chemosis of lids etc. and no evident signs of toxicity were observed.[34, 35]

MATERIALS AND METHODS

Objective: Reactive oxygen species (ROS) are highly involved in the pathogenesis of dry eye disease and topical and oral antioxidant therapy has shown a protective role in preventing the damage. In view of this It is imperative to explore the antioxidant potential of eye drop, as the suppression of Oxidative stress plays an important role in dry eye syndrome besides the management of symptoms due to deficient tear film components. The study aims at *In vitro* biochemical antioxidant screening comprising of Nitric oxide radical scavenging assay, ABTS radical scavenging assay, DPPH radical scavenging assay and Ferric reducing antioxidant potential (FRAP) assay.[36-41]

Determination of antioxidant potential was done adopting the following In *vitro* biochemical assays.

1. **Inhibition of Nitric oxide radical:** Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions. This was measured by the Griess reaction (Green *et al*., 1982; Marcocci *et al*., 1994). The reaction mixture (300µl) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and eye drops and the reference compound in different concentrations (10, 25, 50, 75 and 100 µg) were incubated at 25°C for 150 min. Each 30 min, 50µl of the incubated sample was removed and 50µl of the Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4) were added. The absorbance of the chromophore formed was measured at 546 nm on ELISA plate reader (Bio-Tek). All the tests were performed in triplicate and the results averaged. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples. Ascorbic acid served as a positive control compound.

2. **ABTS radical cation decolourisation assay**: In this assay, the oxidant is generated by persulfate oxidation of 2, 2,-azinobis (3-ethylbenzoline-6-sulfonic acid)-($ABTS^{2-}$) as described by Re *et al*., (1999). ABTS radical cation ($ABTS^{+}$) are produced by reacting ABTS solution (7mM) with 2.45 mM ammonium persulphate and the mixtures were allowed to stand in dark at room temperature for 12-16 hr before use. After 16hr, this solution was diluted with ethanol until the absorbance reaches 0.7 ± 0.02 at 734 nm. For the study, 100µl of eye drops (120µg/ml) were added to 200µl of ABTS solution. The absorbance was read at 745nm and the percentage inhibition calculated.

3. **Inhibition of DPPH radical:** The free radical scavenging activity of eye drops was measured by 1; 1-diphenyl-2-picryl-hydrazil (DPPH) uses the method of Blois (1958) and Gomez-Alonso *et al*., (2003). 0.1 mM solution of DPPH in methanol was prepared and 100 µl of this solution was added to 100 µl of eye drops and the reference compound (50, 100, 150, 200 and 250 µg). After 30 min, absorbance was measured at 517 nm. Butylated Hydroxy Anisole (BHA) was used as the reference material. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.

4. **Reducing power/Ferric reducing antioxidant potential (FRAP) assay:** The reducing power of eye drops was determined according to the method of Oyaizu (1986). 100 µl of eye drops were mixed with phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixture was incubated at 50ºC for 20 min. A portion of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000g for 10 min. The upper layer of the solution (100 µl) was mixed with distilled water (50µl) and Ferric chloride ($FeCl_3$) (100 µl, 0.1%) and the absorbance was measured at 700 nm. Butylated Hydroxy Toluene (BHT) was used as the reference material. All the tests were performed in triplicate and the graph was plotted with the average of three observations.

RESULTS AND DISCUSSION

In vitro biochemical antioxidant assays such as Nitric oxide radical scavenging assay, ABTS radical scavenging assay, DPPH radical scavenging assay and Ferric reducing antioxidant potential (FRAP) assay have confirmed the antioxidant potential of the herbal eye drop. The Inhibitory Concentration (IC_{50}) obtained from the standard graphs of various assays are expressed in terms of their standard compounds (Table-1) (Figure-1, Figure-2, Figure.3, Figure 4).

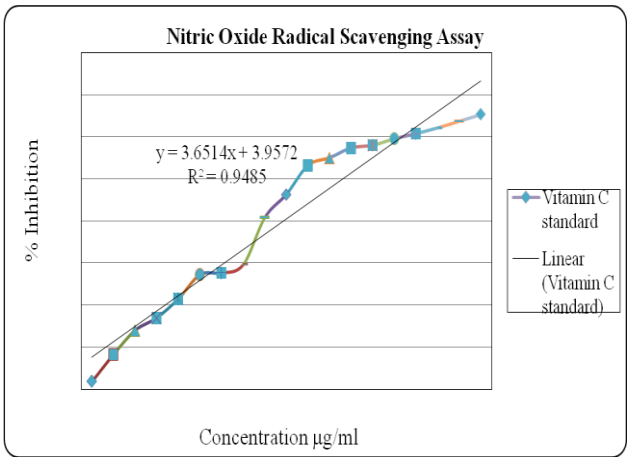

Figure 1: Nitric oxide radical scavenging assay obtained with vitamin C standard.

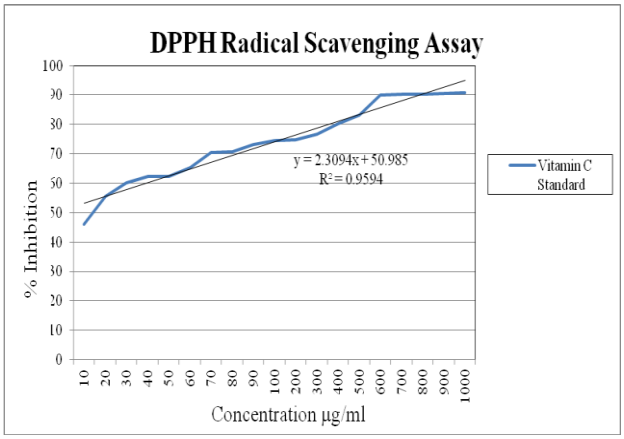

Figure 2. DPPH radical scavenging standard assay obtained with vitamin C standard.

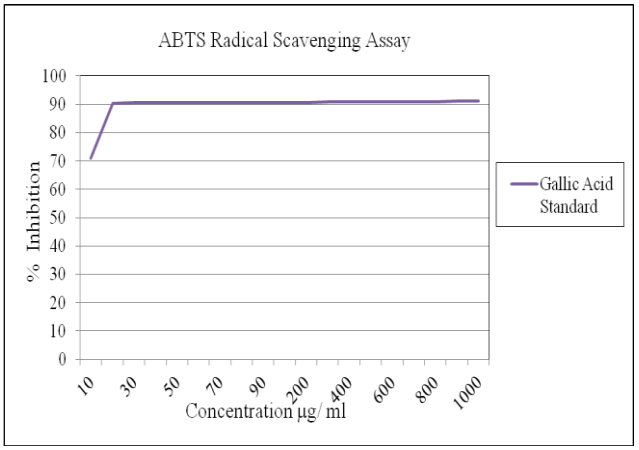

Figure 4: ABTS radical scavenging assay with gallic acid standard.

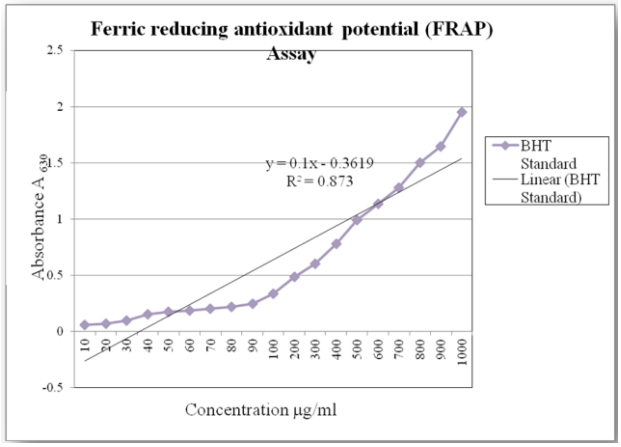

Figure 3: FRAP standard assay obtained with BHT standard.

Table 1: Inhibitory Concentration (IC_{50}) obtained from the standard graphs of various assays are expressed in terms of their standard compounds.

Sl. No.	Results	Sample volume of eye drops solution 120µg/ml	IC_{50} values equivalent to defend Standards
1.	Nitric Oxide Radical Scavenging Assay	100µl	632.99 µg/ml of Vitamin C Standard
2.	ABTS Radical Scavenging Assay	100µl	527.70 µg/ml of Gallic Acid Standard
3.	DPPH Radical Scavenging Assay	100µl	719.32 µg/ml of Vitamin C Standard
4.	Ferric reducing antioxidant potential (FRAP) Assay	100µl	276.69 µg BHT/ml

CONCLUSION

Despite progress in determining the etiology and pathogenesis of dry eye syndrome, current knowledge remains inadequate, and no preventive strategies have been found. The present-day management strategy of dry eye syndrome though clinically effective, poses certain limitations Moreover, the most common therapy for dry eye syndrome, artificial tears, provides only temporary and incomplete symptomatic relief. Hence, identification of modifiable risk factors for dry eye syndrome may suggest avenues for investigation of novel preventive and treatment measures.[42] In this scenario, it is becomes essential to translate some promising leads for management of dry eye syndrome from Ayurvedic literatures into, safe, effective and quality assured dosage forms to improve patient's compliance. In addition to the management of symptoms related to deficiency of tear components, prevention of damage due oxidative stress, arrest of further progress and control of infection also forms an vital component in dry eye disease. The close relationship between ocular surface epithelia and the preocular tear film ensures ocular

surface health. As such, dysfunctional protective elements that lead to ocular surface and tear disorders are heterogeneous, effective therapeutic strategies are the need of hour to tackle tear disorders attributed with diverse factors. The ocular toxicity studies of standardized herbal eye drops revealed its safety on topical ophthalmic use. Further the antioxidant and anti-microbial property may contribute to effective symptom management and extenuation of basic pathology linked with tears component deficiency. The eye drops developed rationally taking potential leads from codified Ayurvedic texts probably contribute by offering comprehensive management for dry eye syndrome.

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ANTIMICROBIAL ASSAYS OF AN AYURVEDIC HERBAL EYE DROPS FOR DRY EYE SYNDROME

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ABSTRACT

Kerato-conjunctivitis sicca (KCS), or dry eye disease, is one of the most common complaints seen by ophthalmologists. In the existing scenario of ageing population and increasing environmental factors it is becoming even more prevalent. Although dry eye is not a trivial complaint, the symptoms cause significant discomfort and substantially reduce the sufferer's quality of life. Together with measures such as tear preservation, use of tear stimulants and tear substitutes, prevention and control of infection also comprehends as an important approach in the management of Kerato-conjunctivitis sicca (KCS), or dry eye syndrome (DES). Considering its importance, antimicrobial assay of the Ayurvedic herbal eye drops formulated for dry eye syndrome was performed. The study encompasses antibacterial assays for determination of Minimum Inhibitory Concentration (MIC) using pathogenic strains of bacteria; *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhinurium*, *Escherichia coli*, *Klebsiella*

pneumonia as test organisms and screening of antifungal activity of eye drops against *Aspergillus fumigatus and Candida albicans.* The studies revealed notable anti-bacterial and anti-fungal activity against selected microbial strains, conceivably compliment to the management of dry eye syndrome.

KEYWORDS: antimicrobial assay, Ayurvedic herbal eye drop, dry eye syndrome.

INTRODUCTION

Dry eye syndrome (DES) or Kerato-conjunctivitis sicca (KCS) is an eye disease caused by eye dryness, which, in turn, is caused by either decreased tear production or increased tear film evaporation.[1] The Latin phrase "kerato-conjunctivitissicca" indicates dryness and inflammation of the cornea and conjunctiva. Ayurvedic literatures describe DES as *shushkakshipaka, parishuskha-netra, ativishuskha--netra, asrusravarahita-netra* and *asnigdha-netra* indicative of dryness of eye due to deficiency in tear film components.[2] There are many conditions which cause dryness of the eyes such as hypo function of lacrimal glands, mucin deficiency, conjunctival scarring etc.[3] Dry eye syndrome is the most common eye disease, affecting 5-6% of the population.[4,5,6] Management strategies of DES encompass mainly supplementation of tear preferably substitutes containing methylcellulose or carboxy-methylcellulose or identical substances which are viscous in nature.[7] The tear stimulants such as cholinergic drugs increases the tear production from lacrimal gland by stimulating secretions, but not been used in clinical practice (Table.1).

Table-1: Contemporary management strategies for Dry eye syndrome and limitations.

Approaches	Modalities and Limitations
Tear Substitutes •***Methyl cellulose*** •***Carboxy methyl cellulose*** •***Poly Viny1 alcohol***	• Preservatives used in formulations are known to cause dry eyes. • All these drugs do not have any effect on basic patho-physiology and they provide only symptomatic relief.
Tear Preservation	• Tear Preservation can be done by occluding the puncta or minimizing evaporation, • Useful as short-term measure to assess the effect of occluding puncta before resorting to permanent measures
Tear Stimulants	• Tear Stimulants such as Cholinergic drugs increases the tear production from lacrimal gland by stimulating secretions. • Not been used in clinical practice.

All these drugs do not have any effect on basic patho physiology and they provide only symptomatic relief.[8]

Management of infection-A Pivotal aspect in Dry Eye Disease

Dry eye disease commonly occurs after an episode of viral kerato-conjunctivitis or severe acute or sub acute conjunctivitis. These diseases may lead to loss of goblet cells from the conjunctival epithelium and release of inflammatory cytokines. Patients usually complain of persistent symptoms and continue to be treated for the original condition. This treatment is not only inappropriate, it may also be toxic; whereas they are actually suffering from the vicious cycle of secondary tear film alterations.

Further, Blepharitis, an extremely frequent cause of dry eye disease, has infectious and inflammatory components It results in impairment of the lipid phase of the tear film and an increased rate of tear evaporation. In addition, dysfunction of the meibomian glands and poor elimination of abnormally thick and viscous lipid secretions provide favorable conditions for secondary bacterial infection at the base of the eyelashes. The toxins released by the bacteria aggravate the condition and produce lesions on the cornea adjacent to the lid margins.

Topical antibiotics and corticosteroids are sometimes used to treat secondary infections and inflammation. But discontinuation of antibiotics, steroids and all preservative-containing eye drops is mandatory for relief of symptoms and progressively improving the tear film and ocular surface.In view of the above, there is an urgent need to evolve safe and effective management approach to tackle symptoms, infections and underlying pathology associated with DES.[24]

Development of Comprehensive therapeutic Approaches-Need of the Hour

Owing to challenges of different conventional agents to manage such instances it is imperative to develop such topical dosage form which could offer safe, effective and comprehensive management. Ayurvedic literatures record more than fifty ophthalmic plant drugs and more than forty metals minerals having diversified pharmacological actions on visual system and adnexa of the eye.[9,10,11] Adding to the references from Ayurvedic texts,few clinical studies on medicinal plants such as Yastimadhu (*Glyeyrrhiza glabra*) and Daruharidra (*Berberis aristata*) also substantiate the usefulness of these plants in surface inflammatory ocular lesions such as dry eye syndrome. A study intervention comprising of topical and internal use of *Daruharidra* (*Berberis aristata*) has shown significant improvement in subjective parameters like dryness, redness, photophobia etc. in DES.[12,13,14]

Pharmacological actions beneficial to ocular system such as *caksusya* (conducive to vision), *netrya* (conducive to adnexa of eye), *netraruja-hara* (analgesic ophthalmic action), netra-*sodhahara* (anti-inflammatory action) *netrakanduhara* (anti allergic action), *vrana-ropana* (wound healing effect),anti-bacterial actions are attributed to these drugs as codified in Ayurvedic literature.[9,15,16] Yastimadhu (*Glyeyrrhiza glabra*) has shown notable anti-inflammatory action attributed to cortisone-like substance present in this plant that helps reduction of inflammation.[17] It is evident that the combination of ingredients viz. Yastimadhu (*Glyeyrrhiza glabra*), Daruharidra (*Berberis aristata*) may play a significant role in restoring the functions of tear film ,prevention of ulceration and related checking inflammatory process, infection and contributory to the comprehensive management of DES . The eye drop is formulated with these two plant ingredients and developed complying quality standards and other safety parameters.[18,19,20] Ocular toxicity and safety studies revealed The eye drops did not cause irritation to ocular mucous membrane of eyes of rabbits such as opacity ulceration of cornea; congestion, swelling, moderate circumcorneal hyperemia; or injection, hemorrhage, gross destruction of iris; redness and other signs of inflammation of palpebral and bulbar conjunctivae; chemosis of lids etc. and no evident signs of toxicity were observed.[21, 22]

MATERIALS AND METHODS

Objective

It is imperative to explore the anti microbial potential of eye drop, as the control of infection plays a crucial role in dry eye syndrome besides the management of symptoms due to deficient tear film components. The objectives of this study comprise antibacterial and antifungal assays of Ayurvedic herbal drop formulated for dry eye syndrome. The assay was carried out for determining the Minimum Inhibitory Concentration (MIC).[23]

A. Antibacterial Assays for Determination of Minimum Inhibitory Concentration (MIC)

Tetrazolium Microplate Microbial viability Assay, a colorimetric assay based on the reduction of a tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) for rapidly determining the susceptibility of pathogenic strains to bactericidal Ayurvedic drugs was carried out as described by Perumal *et.al.*[21] Pathogenic strains of bacteria; *Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhinurium, Escherichia coli, Klebsiella pneumonia* were used as test organisms. The eye drops were tested for their

antibacterial potential and results were noted down. The 200µl of eye drop was mixed with 100µl bacterial culture in nutrient broth and then inoculated in the 96 well plates.

Serial Dilutions were performed according to the protocol and kept for incubation at 37°C for overnight. The drug-free controls (i.e. only bacterial strain) and appropriate blanks (i.e. only eye drops) were included as negative control. After the overnight incubation, cold 20% Tetrazolium solution was added to each well. The colour change was observed and noted for determining the MIC value of respective drug against the bacterial cultures. The bacterial growth was corresponded with the colour change to pink from the original colour of the respective drug and in absence of growth the colour remained the same. The MIC value was determined by observing the pink colour that indicates bacterial growth (+) and colorlessness that indicates inhibition of bacterial growth (-). The minimum concentration of the drug corresponded to the growth inhibition was treated as the 'MIC'.

B. Antifungal assay

Screening of antifungal activity of eye drops, was carried out against *Aspergillus* sp., *Aspergillus fumigatus and Candida albicans* obtained from Microbial Culture Collection, National Centre for Cell Science, Pune, (NCCS). The drug and the fungal culture in the Sabouraud dextrose broth were mixed in the 96 well plate. Dilutions were performed according to the protocol and kept for incubation at 37°C for 3 days. Each day the fungal growth was observed to determine the MIC value of respective drug against three fungal pathogenic strains.[23] The MIC value was determined by observing the fungal plaques that indicated growth (+) and their absence indicated no growth (-).

RESULTS AND DISCUSSION

A. Observations of Antibacterial Assays

The MIC values of the various concentrations of eye drops against different strains of bacteria were assessed. After overnight incubation, cold 20% tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5- phenyltetrazolium chloride (INT) was added and changes in colour were recorded**.** Eye drops have demonstrated antibacterial activities against all 5 bacterial strains under study; except *Bacillus subtilis*, the best effect was against *E coli* (Table.2) (Fig.1).

Table 2: Layout and observations made in antimicrobial assay carried out on 96 microwelltitre plate.

Concentration Eye Drop (µg/ ml) →		**120.0 µg/ ml**	**60.0µ g/ml**	**30.0µ g/ml**	**15.0µ g/ml**	**7.50µ g/ml**	**3.75µ g/ml**	**1.87µ g/ml**	**0.93µ g/ml**	**0.46 µg/ml**	**0.23µ g/ml**	**0.11µg /ml**	**Positive Control**
BLANK	A												
Staphylococcus aureus	B	-	-	-	+	+	+	+	+	+	+	+	+
Bacillus subtilis	C	+	+	+	+	+	+	+	+	+	+	+	+
Pseudomonas aeruginosa	D	-	-	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumonia	E	-	-	-	+	+	+	+	+	+	+	+	+
Escherichia coli	F	-	-	-	-	-	+	+	+	+	+	+	+
Negative control	G	-	-	-	-	-	-	-	-	-	-	-	+
BLANK	H												

(+): Growth of organisms; (-): No growth of organisms.

Antibiotic standard	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Klebsiella pneumonae*	*Bacillus subtilis*
Tetracycline (µg/ml)	10.0	1.25	10.0	0.312	1.25

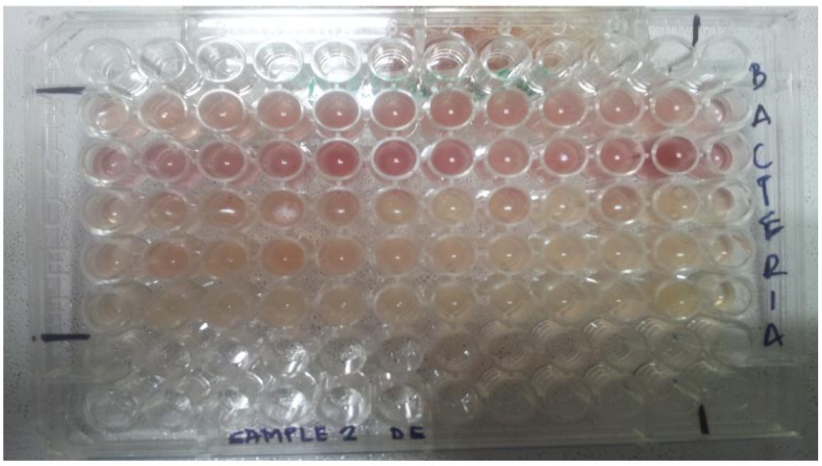

Figure 1: Observations on colour changes after overnight incubation and adding cold 20% tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5- phenyltetrazolium chloride (INT).

B. Observations of Antifungal assay

The MIC values of the various concentrations of eye drops against different strains screening of antifungal activity of eye drops against *Aspergillus spp.*, *Aspergillus fumigatus and Candida albicans* was done. The eye drops at 200 µl volume has antifungal activities against all 3 fungal strains under study (Table.3) (Fig.2).

Table 3: Layout and observations made in antimicrobial assay carried out 96 microwelltitre plate.

Concentration Eye Drop (µg/ml) →		120.0 µg/ ml	60.0 µg/ml	30.0 µg/ml	15.0 µg/ml	7.50 µg/ml	3.75 µg/ml	1.87 µg/ml	0.93 µg/ml	0.46 µg/ml	0.23 µg/ml	0.11 µg/ml	Positive Control
Aspergillus spp.	A	-	+	+	+	+	+	+	+	+	+	+	+
Aspergillus fumigatus	B	-	+	+	+	+	+	+	+	+	+	+	+
Candida albicans	C	-	+	+	+	+	+	+	+	+	+	+	+

(+): Growth of organisms; (-): No growth of organisms.

Standard Antifungal	Minimum Inhibitory Concentration (MIC) in µg/ml		
Amphotericin B	*Aspergillus spp.*	*Aspergillusfumigatus*	*Candida albicans*
	0.156	0.156	0.156

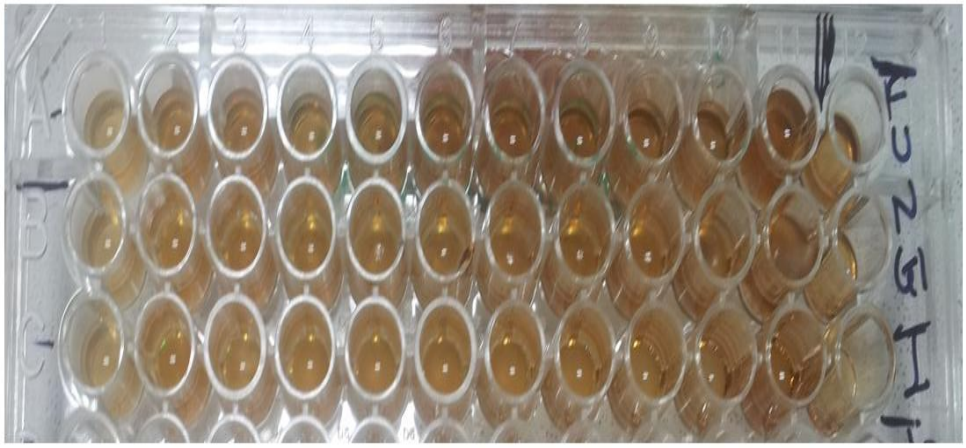

Figure 2: Observations on changes in the turbidity after 72 hrs incubation.

CONCLUSION

In spite of advancements in ophthalmic medicine, several perplexing problems still exist before modern ophthalmologists, demand for special attention to generate tangible evidence and mainstream the practices from ancient medical knowledge. The contemporary management strategy of dry eye syndrome though clinically effective, poses certain limitations. It is pivotal to translate some potential leads for management ofdry eye syndrome as detailed in Ayurvedic texts into user friendly, safe, effective and quality assured dosage forms to improve patient's compliance. In addition to the management of symptoms related to deficiency of tear components, the control of infection also forms an essential constituent in dry eye disease. The close relationship between ocular surface epithelia and the preocular tear film ensures ocular surface health. Dysfunctional protective elements that can lead to ocular surface and tear disorders are heterogeneous, and these different disorders themselves can manifest dysfunctional protective elements. Effective therapeutic strategies need be formulated to manage ocular surface and tear disorders presenting with diverse etiology. The ocular toxicity studies of standardized herbal eye drops revealed its safety on topical ophthalmic use. Further the anti-microbial property may compliment to symptom management and mitigation of underlying pathology linked with tear component deficiency. The eye drops formulated rationally with Ayurvedic herbal ingredients may contribute significantly by offering comprehensive management for dry eye syndrome.

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